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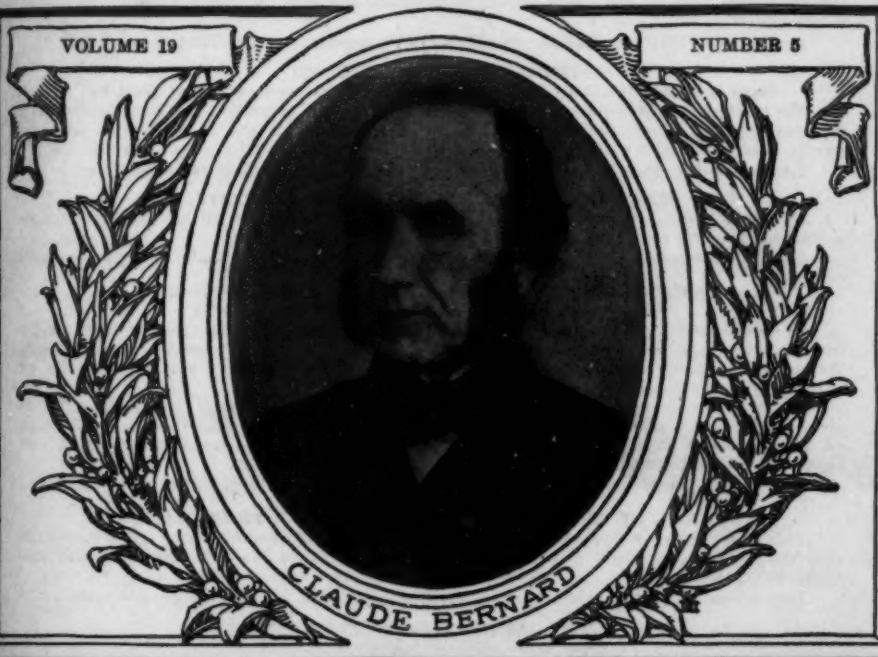

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COMPOSITION OF SOME COMMON FOODS WITH RESPECT TO THE CARBOHYDRATE CONTENT

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(Received for publication January 5, 1940)

In a study in this laboratory on the changes in carbohydrate combustion by a man following ingestion of common foods containing approximately 25 gm. each of carbohydrates, analyses were made of the composition of these foods. In these analyses, supplementing the calculation of the total carbohydrates by difference, determinations were made of the content of reducing sugars, hydrolyzable sugars, and starch. As not all the kinds of food used in this metabolism study (Carpenter, '40) had been previously analyzed in this way and as some of the results differ from the analyses of these foods already published, the values obtained are presented here for purposes of record.

METHODS OF ANALYSIS

In the metabolism experiments, the weight of food to be given to the subject, to contain at least 25 gm. of total carbohydrates, was estimated from the previously published analyses. At the time of each experiment the food was made ready for cooking, if necessary. White potatoes, for example, in amount exceeding the calculated weight, were boiled until soft enough for serving. All visible water was then drained as rapidly and as completely as possible from the potatoes, the portion to be eaten was weighed quickly on a Sauter balance (to 0.1 gm.) and one or two portions were weighed separately as samples for analysis. These samples were dried in an oven at 60°C. until they had lost enough moisture so that they could

be ground for analysis. The loss in moisture was determined before grinding, and the water remaining in the sample was also determined by further drying of a small portion of the air-dry sample after it had been ground. The foods were served as promptly as possible after they were cooked and, therefore, contained the maximum amount of moisture. Hence the amounts of carbohydrates in the food portions given to the subject, although estimated from previously published analyses to be 25 gm., were in most instances noticeably lower.

The usual procedures were used for the determinations of nitrogen, fat, and ash. In the calculation of protein, the factor 6.25 was used, to make the results comparable with earlier analyses, although it is recognized that in some instances other factors should be employed. Solutions of glucose and sucrose were prepared and the starch was hydrolyzed in samples of 1 to 3 gm. of each food, according to standard procedures (Association Official Agricultural Chemists, '35). The amounts of the final reducing sugars in the solutions prepared by the several procedures were determined by the method of Folin ('34). To determine the accuracy of the amounts of reducing sugars, hydrolyzable sugars, and starch recovered by the procedures above mentioned, analyses of mixtures of pure glucose, cane sugar, and starch were made.

The cellulose content was determined by the method of Crampton and Maynard ('38) and the heat of combustion by the oxy-calorimeter (Benedict, '29).

RESULTS

The results of the analyses are given in table 1, the order of listing of the foods being the same as will be given later (Carpenter, '40) concerning the metabolism study with these foods. The percentages of the different constituents and the heats of combustion are given on the basis of the fresh food, in the condition as served to the subject.

The rice and the macaroni were characterized by a lack both of reducing and hydrolyzable sugars. The macaroni had a higher percentage of protein than the rice, but the starch con-

TABLE 1

Composition and energy values of foods as served.

FOOD	DATE OF USE	WATER	PROTEIN	ETHER EXTRACT	ASH	CARBOHYDRATES				UNDETERMINED	HEAT OF COMBUSTION PER GRAM
						Reducing sugars	Hydrolyzable sugars	Starch	Cellulose		
	1937–1938	%	%	%	%	%	%	%	%	%	*kg.-cal.*
Rice, boiled		75.4	2.0	0.02	0.09	0.0	0.00	23.4	0.60	–1.5	1.00
Macaroni, boiled		71.6	4.5	0.05	0.16	0.0	0.00	23.7	0.34	–0.35	1.22
Bread, white, without crusts	Feb. 18	38.9	9.5	1.7	0.78	3.1	0.40	42.5	1.5	1.6	2.58
	Mar. 16	39.0	9.0	0.98	0.48	3.1	0.26	43.6	1.4	2.2	2.60
	Mar. 21	38.5	9.8	1.7	0.73	2.5	0.00	45.1	2.0	–0.33	2.68
Potatoes, sweet, boiled	Oct. 6	65.3	1.6	0.27	0.75	5.2	5.4	13.8	1.6	6.1	1.34
	Jan. 24	71.6	1.2	0.20	0.66	4.0	6.3	11.4	1.6	3.0	1.12
	Feb. 28	75.6	0.90	0.21	0.60	4.3	2.7	10.8	1.6	3.3	0.96
Potatoes, white, raw	Jan. 28	75.9	1.3	0.11	0.72	0.72	0.67	20.8	0.66	–0.88	0.94
	June 6	79.3	2.0	0.10	0.64	0.00	0.00	18.4	0.79	–1.2	0.82
Potatoes, white, boiled	Oct. 4	81.1	1.8	0.04	0.65	0.29	0.00	16.4		–0.28 [1]	0.75
	Feb. 23	81.8	1.6	0.07	0.48	1.01	0.00	15.0	0.59	–0.55	0.73
Peas (fresh), boiled	May 27	81.7	5.2	0.48	0.50	0.00	2.3	7.0	3.6	–0.78	0.83
	June 1	84.2	4.8	0.62	0.48	0.26	1.0	5.4	2.2	1.04	0.73
Parsnips, boiled	Oct. 13	78.6	1.7	0.54	0.70	1.3	3.1	10.8	1.9	1.4	0.90
	Jan. 17	85.9	1.6	0.72	0.51	1.1	3.4	2.6	2.2	2.0	0.59
	Mar. 4	90.1	1.6	0.60	0.56	1.0	1.1	1.3	1.8	1.9	0.44
Beets, boiled	Dec. 20	92.2	1.2	0.21		0.36	2.8	0.83		2.4 [2]	0.30
	Jan. 12	92.2	1.2	0.18	0.48	0.43	2.3	0.99		2.2 [1]	0.31
	Feb. 25	91.1	1.4	0.17	0.49	0.40	3.6	0.90	1.4	0.54	0.35
Carrots, raw	Jan. 26	91.5	0.66	0.40	0.52	1.4	2.4	0.98	1.5	0.64	0.35
	Jan. 31	90.7	0.79	0.40	0.52	0.93	2.5	1.1	1.7	1.4	0.37
	Mar. 7	90.9	0.67	0.19	0.49	1.8	3.0	1.2	1.4	0.35	0.37
Carrots, boiled	Oct. 8	92.2	0.79	0.22	0.43	1.0	2.0	1.3		2.1 [1]	0.32
	Jan. 10	91.8	0.69	0.40	0.42	1.2	2.0	1.3	1.6	0.59	0.33
	June 3	89.9	0.72	0.60	0.51	1.5	3.1	1.4	1.6	0.67	0.41
Squash, boiled	Dec. 22	89.2	1.7	1.2	0.44	2.6	0.96	2.3	1.6	0.0	0.53
	Jan. 21	95.2	0.34	0.18	0.25	1.4	0.07	0.50		2.1 [1]	0.21
	Mar. 2	91.8	0.96	0.58	0.41	1.7	0.18	0.99	2.0	1.4	0.34
Cashew nuts, raw, unsalted	Apr. 20	3.75	19.9	47.6	2.4	0.0	6.0	13.9	1.7	4.8	6.82
cooked, salted	Mar. 23	3.47	18.1	49.9	2.5	0.0	5.9	20.0	1.3	–1.2	6.94
cooked in cocoanut oil	June 17	1.81	20.0	50.7	2.5	0.0	7.3	15.2	2.3	0.19	7.10
Peanuts, roasted, buttered salted	Mar. 31	3.15	26.5	51.8	2.3	0.0	4.3	12.1	4.0	–4.2	7.05
Almonds, blanched	Apr. 6	3.82	21.9	55.9	3.0	0.0	5.8	8.5		1.1 [1]	7.25
Pecans, raw, unsalted	Apr. 8	2.62	11.0	69.7	1.6	0.0	2.5	7.9		4.7 [1]	8.21
roasted, buttered, salted	Mar. 25	2.25	10.4	72.3	1.6	0.0	3.3	6.0	3.1	1.1	8.20
Walnuts, California, raw	Mar. 28	3.19	17.8	66.5	1.8	0.0	2.1	5.3	4.6	–1.3	7.90
Filberts, raw, unsalted	Apr. 4	3.38	14.9	65.6	2.3	0.0	3.4	5.9	4.7	–0 18	7.91
roasted, unsalted	Apr. 15	1.55	14.1	66.6	2.3	0.0	5.8	6.8	3.9	–1.1	7.99
Dates, dried, pitted		19.6	1.8	0.35	1.4	67.4	0.0	3.1	3.1	3.3	2.92
Figs, pressed, Smyrna	Apr. 27	27.3	3.1	0.34	1.8	45.7	7.9	2.9	6.5	4.5	2.73

[1] Including cellulose.
[2] Including cellulose and ash.

tent of both was practically the same. The white bread contained significant amounts of reducing sugars and some hydrolyzable sugars, but mainly starch; the protein content averaged over 9%.

The boiled sweet potatoes and all the other cooked vegetables were characterized by a high water content. The lowest water content was found with one of the samples of sweet potato and the highest with one of the samples of squash. The sweet potatoes contained a significant amount of reducing sugars, varying amounts of hydrolyzable sugars, and from 11 to 14% starch.

The raw white potatoes, in addition to water, contained for the most part starch. In one sample there were no sugars of any kind and in the other sample a total of only 1.4%. The boiled white potatoes had a somewhat lower content of starch and a slightly higher content of water than did the raw white potatoes. The boiled fresh peas were characterized by a higher protein content than any of the preceding foods except the white bread, and had some hydrolyzable sugars and a significant amount of starch.

In the boiled parsnips there were appreciable amounts of the three classes of available carbohydrates, although these varied considerably, particularly the starch content. This variability in starch content was apparently related to the amount of water present, as the sample with the largest amount of starch had the lowest water content. The boiled beets likewise had noticeable amounts of reducing and hydrolyzable sugars, particularly the latter, and just under 1% starch.

The raw carrots all had significant amounts of the three classes of available carbohydrates, the content of hydrolyzable sugars being the highest, that of reducing sugars next, and that of starch the lowest. The composition of the boiled carrots was not materially different from that of the raw carrots except for the fat content, which averaged a little higher in the former. The boiled squash was one of the most variable of the vegetables in composition, as the water content of the

three samples ranged from 89 to 95% and there was a wide variation in the protein and the fat content.

The nuts were characterized by a low water and a high protein content, the latter ranging from 10.4% in the roasted, buttered pecans to 26.5% in the roasted peanuts. The fat content also was high, as it varied from 47.6% with the raw cashew nuts to 72.3% with the roasted pecans. There were no reducing sugars in any of the nuts, but hydrolyzable sugars were found, ranging from 2.1 to 7.3%. The starch content ranged from 5.3% with raw California walnuts to 20.0% with cooked, salted cashew nuts.

The dried figs and dates were characterized by high amounts of reducing sugars. No hydrolyzable sugars were found in the dates, but there was 8% in the figs. Slight amounts of starch were found in both fruits.

On the fresh basis, the cellulose content was lowest with boiled macaroni and highest with one of the samples of peas, some of the nuts, and the two fruits. The nuts, however, had relatively small amounts of moisture. When the values are calculated to a water-free basis, the differences in the amounts of this constituent are more evident. The rice, macaroni, bread, white potato, and most of the nuts have under 5% cellulose on the water-free basis, the vegetables other than sweet and white potatoes contain from 9 to 24% (the majority over 15%), and the dates and figs have 4 and 9%, respectively.

The amount of undetermined material on the fresh basis was low for the most part, the highest value being 6.1% with sweet potato. In a number of instances the sum of the determined constituents exceeded 100%, which indicates a summation of errors in the several determinations. In these instances the percentages of undetermined material have been given minus signs in table 1. On the water-free basis, the highest values are with boiled sweet potatoes (11 to 18%), boiled parsnips (7 to 20%), raw carrots (4 to 15%), boiled carrots (7 to 8%), and the third sample of squash (16%). Most of the values for the other foods are under 5%. In the vegetables some material was left undetermined by the conventional methods. Lignin was not determined.

The heats of combustion of the various foods per gram of fresh material varied widely. The cooked vegetables had the lowest values. The boiled beets, raw carrots, two of the samples of boiled carrots, and two of the samples of squash had energy contents of under 400 gm.-calories. For the boiled peas and the white potatoes, both cooked and raw, the values approach nearly 1000 gm.-calories. Of the boiled vegetables, the sweet potatoes had the highest energy content. The nuts, because of their high protein and fat content and low water content, had high energy values, ranging from 6.82 kg.-calories with raw cashew nuts to 8.21 kg.-calories with raw pecans.

When the undetermined material is assumed to have a caloric value of 4 kg.-calories per gram (fresh basis) and the calories in the undetermined material thus calculated are added to the calories calculated for the other known constituents, the agreement between the calculated total caloric value of the food and the caloric value as determined by the oxycalorimeter is, for the most part, good. The greatest discrepancy is 10%, noted with the boiled squash (see "January 21" in table 1). Most of the other differences range under 5%.

COMPARISON WITH PREVIOUS ANALYSES

In the calculation of the amount of food to be given to the subject to supply 25 gm. of carbohydrates, several sources of data were consulted as a theoretical basis.[1] These were the compilation of analytical results made by the United States Department of Agriculture (Atwater and Bryant, '02), and the reports of McLester ('28), McCance, Widdowson and Shackleton ('36), and Mitchell and Beadles ('37). To compare the results in table 1 with these previous analyses, the water

[1] Olmsted and Williams ('39) have reported that by their method of analysis they have been able to account for more than 98% of the carbohydrate in a variety of common foods, but they give no tabulated results.

content in the several foods must be known, because the amount of water in the material determines the general level of the other constituents.

The water content was essentially the same as reported in earlier studies in the case of boiled rice, raw white potato, raw and cooked carrots, and all the nuts, lower in boiled macaroni, and higher in the other foods. When the other constituents are calculated on the basis of water-free material, comparison shows that the percentages of total carbohydrates were approximately the same in white bread, raw white potato, peas, raw carrots, the nuts, and the fruits as in these same types of food previously analyzed. The values for total carbohydrates in squash were variable and not comparable with values reported previously. They were higher in rice, macaroni, sweet potato, and beets, and lower in the other foods.

The distribution of carbohydrates in the sweet potatoes, the raw white potatoes, and the green peas was not materially different from earlier findings. The parsnips contained reducing sugars whereas the earlier analyses showed none, but the content of hydrolyzable sugars was about the same in both instances. The beets contained a smaller percentage of hydrolyzable sugars, the raw and cooked carrots a smaller percentage of reducing sugars, and the cooked carrots nearly the same percentage of hydrolyzable sugars as previously reported.

The green peas contained a larger proportion of protein and the parsnips about the same proportion. There was some starch in the raw carrots whereas none was found in the previous analyses. The starch content was greater in the boiled carrots and the squash. The protein and fat content of the nuts and the dried fruits were much the same as reported before.

The chemical analyses of the foods were made by Mr. Martin Stankard and the heats of combustion by Mr. Basil James.

SUMMARY

Twenty kinds of common foods, including rice, macaroni, white bread, vegetables (raw and cooked), nuts, dates, and figs, were analyzed with special reference to their content of reducing sugars, hydrolyzable sugars, starch, and cellulose. Determinations were also made of the water, protein, fat, and ash content, and the heats of combustion. The results obtained are compared with previous analyses of similar foods by other investigators.

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THE COMBUSTION OF CARBOHYDRATES IN MAN AFTER INGESTION OF COMMON FOODS

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FIVE FIGURES

(Received for publication January 5, 1940)

Many studies have already been made of the respiratory exchange and the combustion of carbohydrates after ingestion of various types of pure sugars, either singly or in combination. However, the foods commonly eaten by man (table sugar or sucrose, maple sugar, and honey excepted) do not consist of pure sugars alone but contain the more complex carbohydrates and other materials such as protein, fat, ash, and varying amounts of water. Man's main sources of energy from carbohydrates are the sugar in milk and the sugars and starches in fruits, cereals, and vegetables. The rapidity with which carbohydrates in these foods are burned in the body after ingestion and the amounts burned may vary greatly. A special study was therefore made of the combustion of carbohydrates by man after ingestion of various common foods that contain not only the simple sugars but the more complex carbohydrates. The object of this study was to secure information as to how quickly a certain amount of carbohydrates in various foods would be available for combustion in the body and how much carbohydrate would be burned in a given time.

EXPERIMENTAL

The subject was a man (age, 23 years; height, 176 cm.; initial weight, 65.8 kg.; final weight, 70.6 kg.), a medical student, apparently in good health. His respiratory exchange

was measured by the open-circuit helmet respiration apparatus (Benedict, '33). After a preliminary 15-minute period of breathing in the helmet, there were three 15-minute base line (sitting, post-absorptive) periods of measurement, followed by an interval during which the subject ate the food (also drinking 250 cc. of water at room temperature), and then by twelve successive 15-minute periods. All experiments were in the morning, usually ending between 1 P.M. and 2 P.M. The subject voided urine upon arrival at the laboratory, about 8 A.M., and again after the experiment ended. The partition of the carbohydrate, fat, and protein combustion in the total metabolism was calculated in the conventional manner, the protein combustion being estimated from the grams of nitrogen (multiplied by the factor for body protein, 6.0) eliminated in the second voiding of urine.

Analyses of the ventilating air current of the helmet apparatus were made with the gas-analysis apparatus of Carpenter ('33). Fifty-nine analyses of outdoor air gave an average of 0.032% for carbon dioxide and 20.940% for oxygen, with standard deviations of 0.0015 and 0.0043 for the two gases, respectively. Fifteen analyses of air from the helmet when alcohol was burned in it gave an average respiratory quotient of 0.670, with a standard deviation of 0.0071.

RESULTS

Foods eaten and amounts to give 25 gm. of available carbohydrates. It was planned to give such an amount of food in edible condition as would contain a total of 25 gm. of available carbohydrates. The amounts of the different foods necessary for this purpose were calculated from previous analyses made by other investigators. At the time of each metabolism experiment, a sample of the particular food served was saved for subsequent analysis. The results of these analyses have been given in a preceding publication (Carpenter, '40). As there pointed out, because of the quickness of serving the foods after they were cooked, the water content was greater

in most instances than found in the previous analyses. Hence the available carbohydrates in the foods as eaten (fifth column, table 1) were in most instances under 25 gm. Subsequently calculations were made (based upon the actual analyses of the foods) of the amounts of the foods that should have been eaten in order for the subject to get 25 gm. of available carbohydrates. These calculated amounts differ greatly. For raw white potato and raw carrots the amounts were somewhat less than those of the same vegetables when cooked, perhaps because soluble carbohydrates leached from the vegetables as they were cooking or because the vegetables took on water while boiling. The amounts of dried dates and figs were but little more than those of cane sugar and glucose.

Increases in carbohydrate combustion during 3 hours after food ingestion. In table 1 are also recorded the carbohydrate combustion of the subject when post-absorptive (base line), the increases in carbohydrate combustion above the base lines as measured during the 3 hours immediately following ingestion of the foods, and the calculated increases that might have occurred in this time, had each food portion contained 25 gm. of available carbohydrates. In this calculation it was assumed that the ratio between the increase in combustion as measured and the theoretical increase after ingestion of 25 gm. of available carbohydrates would be the same as the ratio between the available carbohydrates in the food as eaten and 25 gm. The following discussions deal with the increases as calculated on this basis. In three of the four water experiments the increases in carbohydrate combustion were over 5 gm., indicating that water alone actually increases the combustion. In several of the food experiments the increases were no greater than after water alone and may have been the result wholly of the water drunk when the foods were eaten. No allowance has been made in any of the results for increase caused by water alone, as it is uncertain whether there is a summation of effects when dry food and water are consumed at the same time. With glucose the increases were much the same as with cane sugar. With dates and figs they

TABLE 1

Carbohydrate combustion as influenced by ingestion of 25 gm. of available carbohydrates in common foods

DATE OF EXPERIMENT	MATERIAL			AVAILABLE CARBOHYDRATES IN FOOD EATEN	CARBOHYDRATE COMBUSTION PER 3 HOURS		
						Increase after food	
	Kind	Amount eaten	Amount calculated to give 25 gm. available carbohydrates		Base line	As measured	Calculated for 25 gm. available carbohydrates in food
1937–1938		*cc.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Oct. 15 [1]	Water	250			10.6	5.9	
Dec. 15 [1]		250			8.4	5.2	
Feb. 11 [1]		250			18.8	0.0	
Mar. 11 [1]		250			6.8	6.5	
		gm.					
Oct. 11	Glucose	25	25	25	13.9	7.7	7.7
Jan. 3		25	25	25	8.4	9.7	9.7
June 9	Cane sugar	25	25	25	10.7	10.3	10.3
June 15		25	25	25	15.9	7.5	7.5
June 13 [1]	Potato starch, uncooked	25	25	25	14.0	2.8	2.8
June 10 [1]	Potato starch, cooked	25	25	25	12.5	5.7	5.7
Jan. 7	Rice, boiled	106.8	106.8	25	19.2	5.6	5.6
Jan. 19		106.8	106.8	25	13.5	5.5	5.5
Mar. 14		106.8	106.8	25	15.1	6.4	6.4
Dec. 27	Macaroni, boiled	118.9	105.4	28.2	13.1	6.2	5.5
Jan. 14		118.9	105.4	28.2	20.9	5.0	4.4
Mar. 9		118.9	105.4	28.2	13.0	6.4	5.7
Feb. 18	Bread, white	45	54.3	20.7	8.3	9.0	10.9
Mar. 16		45	53.3	21.1	16.3	5.4	6.4
Mar. 21		45	52.6	21.4	17.2	5.8	6.8
Oct. 6	Potatoes, sweet, boiled	60	102.7	14.6	14.8	7.3	12.5
Jan.24		61	115.5	13.2	16.8	5.2	9.8
Feb. 28		61	141.2	10.8	17.5	4.5	10.4
Jan. 28	Potatoes, white, raw	136	112.6	30.2	16.6	5.0	4.1
Feb. 7		136	124.3	27.6	16.8	3.8	3.4
June 6		136	136.0	25.0	16.9	2.6	2.6
Oct. 4	Potatoes, white, boiled	116	150.3	19.3	10.7	7.8	10.1
Dec. 17		121	152.8	19.8	15.1	10.9	13.8
Feb. 23		118	136.4	18.2	5.0	7.3	9.7
June 1		200	370.4	13.5	7.8	7.7	14.3
Oct. 13	Parsnips, boiled	225	164.5	34.2	20.5	11.0	8.0
Jan. 17		225	353.8	15.9	14.7	10.3	16.2

Dec. 17		121	152.8	19.8	15.1	10.9	13.8
Feb. 27		118	130.4	18.2	5.0	7.3	9.7
June 1		200	370.4	13.5	7.8	7.7	14.3
Oct. 13	Parsnips, boiled	225	164.5	34.2	20.5	11.0	8.0
Jan. 17		225	353.8	15.9	14.7	10.3	16.2
Mar. 4 [1]		225	730.5	7.7	8.7	12.2	39.6
Dec. 20	Beets, boiled	272	618.2	11.0	13.4	10.7	24.3
Jan. 12 [1]		275	680.7	10.1	19.6	5.6	13.9
Feb. 25		275	509.3	13.5	10.7	9.9	18.3
Jan. 26	Carrots, raw	275	524.8	13.1	15.0	8.1	15.5
Jan. 31		275	554.4	12.4	16.3	11.7	23.6
Mar. 7		275	414.2	16.6	10.0	11.2	16.9
Oct. 8	Carrots, boiled	323	572.7	14.1	11.4	11.5	20.4
Jan. 10		325	560.3	14.5	16.0	7.5	12.9
June 3		325	421.0	19.3	9.2	10.9	14.1
Dec. 22	Squash, boiled	257	428.3	15.0	13.7	12.3	20.5
Jan. 21 [1]		257	1285.0	5.0	10.3	4.7	23.5
Mar. 2		257	856.7	7.5	14.3	7.4	24.7
Apr. 20	Cashew nuts, raw	120	125.5	23.9	10.4	7.8	8.2
Apr. 22	Cashew nuts, raw	120	125.5	23.9	8.2	6.3	6.6
Mar. 23	Cashew nuts, cooked, salted	105	96.5	27.2	8.6	6.9	6.3
June 17 [1]	Cashew nuts, cooked in cocoanut oil	105	110.8	23.7	17.7	2.6	2.7
Mar. 31	Peanuts, roasted, buttered	150	151.8	24.7	11.7	5.6	5.7
Apr. 6	Almonds, blanched	150	174.4	21.5	10.3	1.1	1.3
Apr. 8	Pecans, raw, unsalted	200	241.5	20.7	14.3	2.4	2.9
Mar. 25	Pecans, roasted, buttered, salted	200	270.3	18.5	14.2	1.6	2.2
Mar. 28	Walnuts, California	200	335.6	14.9	15.3	2.3	3.9
Apr. 13	Walnuts, California, raw	200	335.6	14.9	17.2	3.3	5.5
Apr. 4	Filberts, raw, unsalted	200	270.3	18.5	12.3	4.2	5.7
Apr. 15	Filberts, roasted	200	198.4	25.2	13.1	4.1	4.1
Apr. 25	Dates, dried	35	35.4	24.7	13.7	10.4	10.5
Apr. 29	Dates, dried, pitted	35	35.4	24.7	14.3	9.7	9.8
Apr. 27	Figs, pressed (Smyrna)	34	44.3	19.2	12.9	9.2	12.0

[1] The results of these experiments were omitted from the average values plotted in figures 1 to 5.

were somewhat greater. The uncooked potato starch did not result in as great an increase as the cooked potato starch nor even as great as the water alone. With boiled rice and macaroni, the increases were similar to those with water alone. When raw white potatoes were eaten, the effect on the carbohydrate combustion was not so great as the effect of water alone. Decidedly larger increases occurred after the ingestion of boiled than raw white potato, but a slightly smaller increase, on the average, was found after eating boiled as contrasted with raw carrots. It is generally believed that cooking of vegetables increases their digestibility. The experiments with carrots indicate that there is considerable absorption of the carbohydrate content in the raw form, but those with white potatoes show only a small absorption in this form. The difference between these two raw foods is probably ascribable to the difference in the nature of the carbohydrate content, carrots having more soluble carbohydrates than starch, and white potatoes having predominantly starch. In half of the instances the nuts resulted in smaller increases than did water alone. The boiled vegetables that may be characterized as sweet (parsnips, beets, carrots, and squash) caused the greatest increases. The nuts and the foods rich in starch, like rice, macaroni, white potato, and bread, gave the smallest increases. When dates, cane sugar, and glucose were eaten, the increases were only about two-thirds of those noted after beets and carrots.

Increases in carbohydrate combustion compared with character of food constituents. The increases in carbohydrate combustion were related to some extent to the amounts of reducing sugars in the several foods. When there were no reducing sugars in the foods, the increases per 3 hours were low, except for cane sugar. When the foods contained up to 2 gm. of reducing sugars, the increases were higher. When they contained between 2 to 4 gm., the increases were still higher, but when they contained from 5 to 17 gm., the increases averaged about the same as in the 2- to 4-gm. group. The foods having from 20 to 25 gm. of reducing sugars, on the

contrary, caused increases that were not much greater than those which occurred with the foods having only up to 2 gm. The increases were, in general, smaller with the foods that contained no hydrolyzable sugars, although the relationship was not marked. The smaller the starch content of the food, the greater was the increase. The carbohydrate combustion was more dependent upon the presence of reducing and hydrolyzable sugars than upon the presence of starch in the foods. Because of the great difference between the fat content of the nuts and the other foods, it is difficult to make a comparison with this food constituent, except to state that the increases in carbohydrate combustion were generally lower with the foods (nuts) having the very high fat content.

Course of carbohydrate combustion after food ingestion. In figures 1 to 5 are indicated the average level of carbohydrate combustion per 15 minutes in the three base line periods and the average levels in the successive 15-minute periods following ingestion of each type of food studied. Each curve represents the average of the results obtained in the several experiments with the particular food (except as indicated by the footnote in table 1), corrected to the basis of a food portion containing 25 gm. of available carbohydrates. The base line carbohydrate combustion in the individual experiments varied from 0.41 to 1.75 gm. per 15 minutes and averaged 1.12 gm. The base line respiratory quotient ranged from 0.75 to 0.85 and averaged 0.80. The changes in respiratory quotient in the successive 15-minute periods after food ingestion closely paralleled the changes in carbohydrate combustion.

There was a marked difference between the availability of glucose and of cane sugar. With cane sugar (fig. 1) there was an immediate maximum rise in carbohydrate combustion in the second quarter hour after ingestion (greater than that noted with any of the other foods), followed by a sharp decrease, an approach to the base line after the first hour, and a return to it at the end of 3 hours. The rise after glucose (fig. 4) was much less marked during the first three 15-minute

periods, and the maximum rise did not occur until the sixth period. Thereafter the carbohydrate combustion decreased gradually, but even at the end of 3 hours it was still appreciably above the base line. Apparently glucose is used first to fill the glycogen reserves of the body, after which it becomes available for combustion, and the combustion lasts a longer time than with cane sugar.

The course of the carbohydrate combustion after ingestion of dates (fig. 1) closely resembled that after cane sugar, the maximum increase occurring in the same period, although it was not so high as after cane sugar and the combustion did not return to the pre-ingestion level at the end of 3 hours. With figs (fig. 2) the maximum increase appeared later than with dates and was not so great, but the subsequent increases were greater.

It has been shown in earlier studies (Carpenter, Bensley, Dill and Edwards, '37) that the initial rise in the respiratory quotient after ingestion of fructose is caused by the formation of lactic acid. It is probable that the early maximum rise in carbohydrate combustion noted both with cane sugar and with dates is also ascribable to the fructose in these two materials.

After rice (fig. 1) there were small increases during the first three periods, the maximum was not reached until the beginning of the third hour, and there was no return to the base line at the end of 3 hours. In one experiment, continued for 4 hours, the combustion was still above the base line at the end of that time. Rice, therefore, is a food whose carbohydrate content is only slowly available to the body, and a long time is required for its combustion.

With beets the increase was immediate, but the maximum did not occur until the sixth period, and the combustion was still at a high level at the end of 3 hours. After white bread (fig. 2) the maximum rise occurred in the fifth quarter hour, following which the combustion gradually decreased, but at the end of 3 hours the base line was not reached. There was a distinct difference between the effects of boiled and raw white potatoes, the maximum rise with raw potatoes being

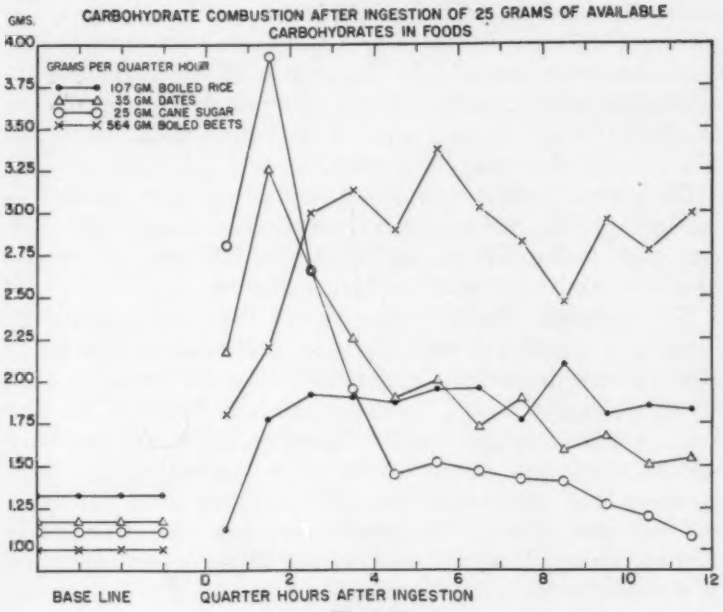

Figure 1

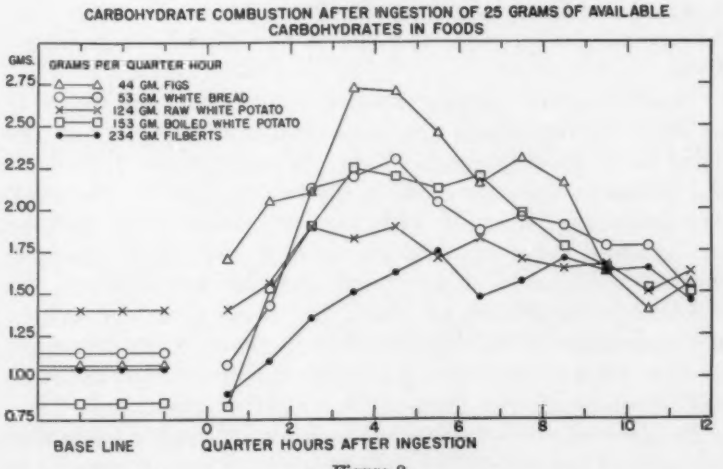

Figure 2

about one-third that with boiled potatoes, although there was not much difference with respect to the time when the maximum rises were found. In the last hour the carbohydrate combustion after ingestion of raw potatoes closely approached the initial level whereas after boiled white potatoes it was still considerably above the initial level.

The increases in carbohydrate combustion were greater in the first hour and the maximum rise occurred sooner after raw than after boiled carrots (fig. 3). In the last hour the results were essentially the same in both instances.

The parsnips (fig. 4) were slowly digested, continually furnishing significant amounts of carbohydrates. The maximum increase in carbohydrate combustion did not occur until the third hour, although a marked rise was found in the first hour. Apparently the readily digestible carbohydrates were burned first, and subsequently more carbohydrates were liberated and made available. With boiled sweet potatoes and with peas (fig. 5) the increase in combustion was nearly over in 3 hours. However, with squash there was no indication of a return to the initial level after 3 hours. With macaroni, although the maximum occurred in the last two periods, there was no great change in the combustion after the fourth period. At the end of 3 hours the combustion was still above the initial level.

The nuts gave varying results, although in the majority of cases the maximum increases appeared early, from the third to the seventh quarter hour. In one instance (almonds) the maximum increase came in the twelfth period. However, the maximum increases with the nuts were small and, on the average, not above 0.85 gm. in any case. When once the maximum increase for any particular nut was attained, the combustion continued at about the same level throughout the remainder of the 3 hours. The increases were somewhat greater after cashew nuts than after the other nuts, although the increases after peanuts were almost as great.

In general, the foods that contained readily available amounts of reducing sugars or hydrolyzable sugars resulted in

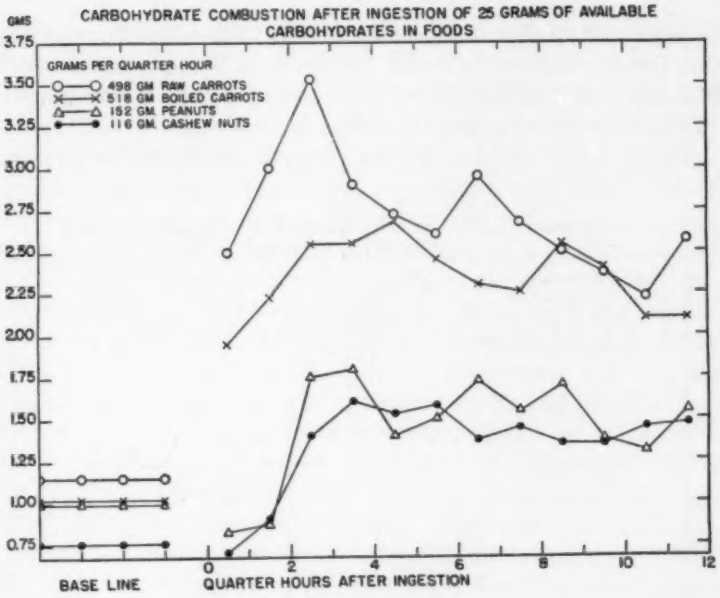

Figure 3

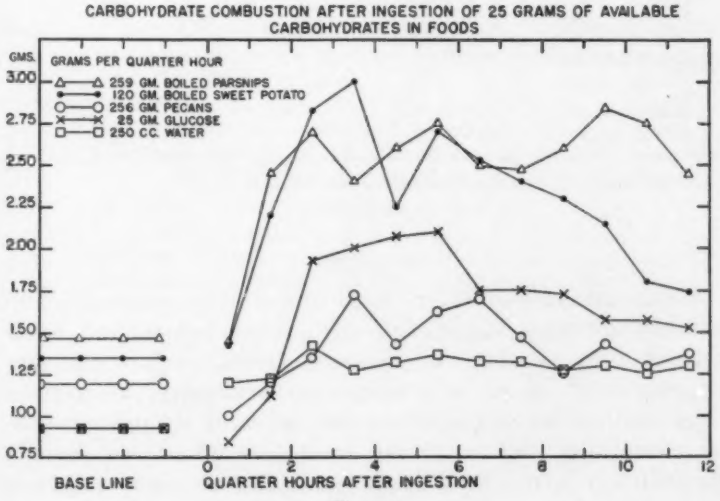

Figure 4

greater and earlier maximum rises in carbohydrate combustion per 15 minutes than did the foods with high starch content and those (nuts) with high fat content, but the increased combustion lasted a shorter time.

The measurements of the respiratory exchange were made by Mr. Basil James.

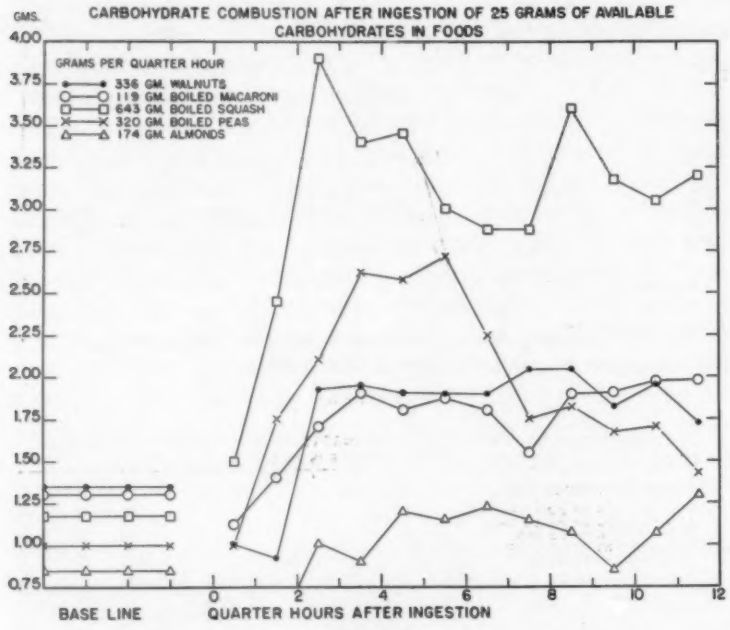

Figure 5

SUMMARY

From measurements of total respiratory exchange and urinary nitrogen elimination of a man before and after ingestion of portions of common foods, each containing approximately 25 gm. of available carbohydrates, calculations were made of the carbohydrate combustion in the post-absorptive condition and in twelve successive 15-minute periods immediately after food ingestion. The amounts required

of the several foods to give 25 gm. of available carbohydrates differed greatly. The increases in carbohydrate combustion during the 3 hours following food ingestion were greater, the greater the amounts of reducing and hydrolyzable sugars in the foods, and smaller the greater the amounts of starch or fat in the foods. The boiled vegetables that may be characterized as sweet (parsnips, beets, carrots and squash) caused the greatest increases. Nuts, rice, macaroni, white potato, and bread caused the smallest increases. The combustion of carbohydrates was greater when carrots were eaten raw than when eaten cooked, but the picture was the reverse with white potatoes. The increase in combustion was sudden and marked but quickly over with cane sugar and dates. That with glucose was slower and less marked but lasted longer. With parsnips the readily digestible carbohydrates were burned first; subsequently the more complex carbohydrates were liberated and made available. With nuts the increases in carbohydrate combustion were small but continuous; they were somewhat greater with cashew nuts than with other nuts.

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CHOLINE METABOLISM

IV. THE RELATION OF THE AGE, WEIGHT AND SEX OF YOUNG RATS TO THE OCCURRENCE OF HEMORRHAGIC DEGENERATION ON A LOW CHOLINE DIET

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ONE FIGURE

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The lack of an adequate amount of choline in the diet of young rats has been found to produce a severely toxic condition to which the name hemorrhagic degeneration has been applied. This deficiency was characterized by the rapid onset of acute renal hemorrhage which could be prevented by the inclusion in the diet of less than one-half the quantity of choline required to avert the formation of a fatty liver. The need for choline was shown to be greater on diets containing added cystine, protein rich in cystine, cholesterol or fat. Methionine exhibited a choline-like action opposite to that of cystine. All of these results were obtained in male rats, 38 to 42 gm. in weight and 22 to 26 days of age (Griffith and Wade, '39 and '40; Griffith, '40). Inasmuch as certain of the experiments suggested a relationship of age or weight to the severity of the ensuing pathological state, a study has been made of the effect of these factors and of sex on the development of this recently recognized result of choline deficiency.

EXPERIMENTAL

The experimental procedures and the constituents of the diet were those previously described (Griffith and Wade, '39). Determinations were made of liver and kidney weights and of

the total chloroform-soluble substances in the liver. The term "liver fat" in tables 1 to 5 refers to this fraction.

The basal diet, AC34, consisted of casein 15%, lard 9, sucrose 62.9, salt mixture 4 (Hawk and Oser, '31), calcium carbonate 1, agar 2, whole dried yeast 6, and the fortified fish liver oil, Natola,[1] 0.1%, respectively. Diets AC32 and AC33 contained 0.3 and 1.0% of added cystine respectively.

The appearance of the kidneys is recorded in tables 1 to 4 as hemorrhagic, partially hemorrhagic, recovered, or normal. The renal hemorrhagic degeneration observed in 40-gm. male rats on diet AC32 resulted in the transformation on the fifth and sixth days of an apparently normal kidney into a greatly enlarged dark red, bloody structure. The mortality was highest on the seventh day. If the animal survived this acute stage the recovery phase was almost as rapid as the degenerative phase. The dark red color of the cortex was replaced by a mottled or blotched light brown appearance, quite distinct from that of the normal kidney. If the hemorrhage was not severe the kidneys were nearly normal, except in size, on the tenth day. In many rats, presumably those more severely affected, the "recovered" kidneys had a frosted appearance due to a white incrustation on the cortex. Kidneys were recorded as normal, if no trace of hemorrhage or of the recovery appearance was evident; as hemorrhagic, if the renal lesion, whether moderate or severe, showed no sign of the recovery process; as partially recovered, if the cortex showed areas of hemorrhage and areas of recovery; and, as recovered, if the cortex showed the mottled surface without any remaining hemorrhagic areas.

RESULTS

Relation of age of 40-gm. male rats to hemorrhagic degeneration. Table 1 shows the relation of the age of the young male rats placed on the choline deficient diet to the severity and duration of the acute phase of this deficiency. The initial

[1] We wish to thank Parke, Davis and Co. for a generous supply of Natola.

weight of these rats was essentially the same, 38 to 42 gm., so that group 1 represented the most rapidly growing and group 8 the least rapidly growing young of this rat colony. These rats were weaned on the twentieth day so that group 1 was placed immediately on the experimental diet and group 8 remained on the stock diet for 7 days before reaching the 40 gm.

TABLE 1

The relation of age to deposition of liver fat and to occurrence of hemorrhagic degeneration in 40-gm. male rats during a 10-day experimental period on a low choline diet

GROUP NO. AND DIET	1 AC32	2 AC32	3 AC32	4 AC32	5 AC32	6 AC32	7 AC32	8 AC32	9 AC33
Age, days	20	21	22	23	24	25	26	27	22
No. of rats in group	43	41	44	44	46	52	40	42	18
Mortality, %	5	24	33	43	37	25	20	17	6
Average final body weight, gm.	57	54	54	50	56	57	57	60	51
Average weight of liver, gm.	4.21	3.96	3.88	3.49	4.06	4.35	4.33	4.64	4.12
Average weight of liver fat, mg.	1140	952	988	745	928	1083	1115	1105	865
Average weight of kidneys (per pair), mg.	924	910	860	892	850	868	882	900	1044
Kidney weight as per cent of normal kidney weight	138	142	134	146	129	130	132	130	171
Appearance of kidneys									
Normal, %	12	10	14	2	9	12	17	19	6
Recovered, %	26	20	16	14	15	29	8	19	22
Partially recovered, %	58	37	25	39	37	25	50	29	33
Hemorrhagic,[1] %	4	33	45	45	39	34	25	33	39

[1] Figures include rats which died during the experimental period.

starting weight. Over 80% of the rats in each group showed evidence of renal pathology on the tenth day. It was probable that most of the remaining 20% were actually "recovered" animals because autopsy of similar rats on the sixth and seventh days showed that 95% or more were moderately or

severely hemorrhagic. Although there was no significant difference in the incidence of the deficiency in these eight age groups, it was evident that the rats in group 1 were less severely affected than the others. Fewer of these rats died during the experimental period and on the tenth day more were in the recovery phase than was true for any of the other groups. The possibility that this difference may have been due to the protective milk diet of the immediately preceding lactation period is being investigated.

The highest mortality, 43%, occurred in rats 23 days of age (group 4). Mortality increased as the starting age of the animals increased from 20 to 23 days of age and then decreased in the older animals. Group 9 shows the effect of increasing the cystine content of the diet from 0.3% (AC32) to 1% (AC33). The hemorrhagic enlargement of the kidneys was intensified, although the mortality was less than that found in the same age group fed diet AC32 (group 3). The usual fatty livers occurred in all of the rats in table 1. Hemorrhage into the eyeball occurred in 20% of these animals.

Relation of age and weight of male rats to hemorrhagic degeneration. The results in table 2 show the occurrence of hemorrhagic degeneration in normal male rats, 24 to 41 days of age, on diets AC32 and AC33. Rats, 65 gm. in weight and 30 days of age, showed the same incidence of the deficiency as the 40-gm. rats reported in table 1. However, the acute stage was not reached until the tenth day (group 3) instead of the sixth day as in the case of the 40-gm. rats (group 1). Recovery occurred in most of these rats by the fifteenth day (group 4).

The effects of choline deficiency were decidedly less evident in rats over 30 days of age (groups 5 to 9). Only 25 to 30% of these older animals showed renal lesions. If renal degeneration occurred, it was moderate rather than severe. None of the animals died. The 10-day period was not too short a period for the production of the deficiency in the older animals because these rats showed fewer signs of renal pathology after 15 days (group 6) than after 10 days (group 5). The use of diet AC33 with its higher concentration of added cystine did

not alter the results (groups 8 and 9). This difference between 30- and 33-day-old rats was unusually interesting because it supported a similar observation noted in the first paper of this series (Griffith and Wade, '39). At that time it was found that the deposition of liver fat in rats over 35 days of age was only

TABLE 2

The relation of age and weight to deposition of liver fat and to occurrence of hemorrhagic degeneration in male rats on a low choline diet

GROUP NO. AND DIET	1 AC32	2 AC32	3 AC32	4 AC32	5 AC32	6 AC32	7 AC32	8 AC33	9 AC33
Age, days	24	30	30	30	33	33	37	36	41
Experimental period, days	6	6	10	15	10	15	10	10	10
No. of rats in group	29	14	20	21	15	16	12	19	21
Average initial body weight, gm.	40	63	65	66	72	71	82	73	88
Average final body weight, gm.	54	83	88	111	107	122	112	103	124
Average weight of liver, gm.	3.76	5.04	5.76	8.33	7.39	9.78	7.53	6.95	8.68
Average weight of liver fat, mg.	988	1060	1572	2275	1968	....	1445	1565	1765
Average weight of kidneys (per pair), mg.	795	797	1288	1217	1050	1202	1155	1082	1385
Appearance of kidneys									
Normal, %	7	93	10	38	73	94	75	63	62
Recovered, %	0	0	0	43	0	6	0	5	10
Partially recovered, %	0	0	15	0	0	0	0	0	0
Hemorrhagic, %	93	7	75	19	27	0	25	32	38

one-half that occurring in rats less than 30 days of age. Apparently there is a marked reduction in the choline requirement of young male rats after reaching 30 days of age.

This relation of age to the occurrence and severity of hemorrhagic degeneration in young male rats is also shown in figure 1. These curves were drawn from data which confirmed in every respect the results noted in tables 1 and 2 on smaller groups of animals. Curve I shows the high incidence of renal

lesions in rats 30 days of age or less and the sharp drop in incidence in rats 33 days of age or older. Curve II shows the per cent of rats with severe renal hemorrhage at the end of the 10-day period. At this time rats under 27 days of age were largely in the recovery phase whereas most of the rats, 30 days of age, were in the acute stage. Curve III shows the per cent of rats which had partially or wholly recovered from the hemorrhagic state. Such recovery was particularly evident in rats 20 days of age. Curve IV shows the mortality in the various age groups. Mortality was highest in rats 23 days of age.

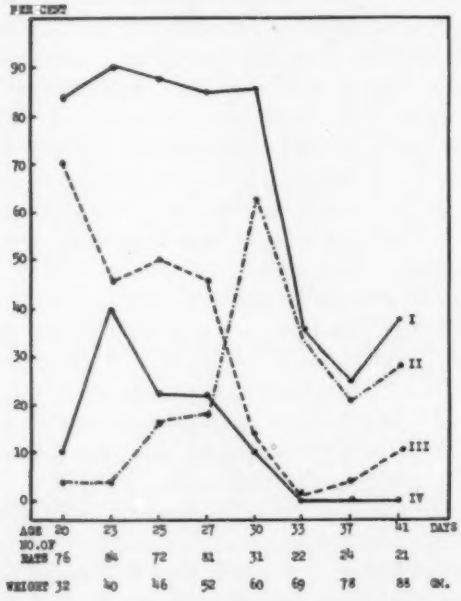

Fig. 1 The relation of age and weight to the occurrence and severity of renal hemorrhagic degeneration in young male rats on a low choline diet (AC32).

Curve I. Per cent of rats showing renal lesions during the 10-day period.

Curve II. Per cent of rats showing hemorrhagic lesions at the end of the 10-day period.

Curve III. Per cent of rats showing partial or complete disappearance of hemorrhagic lesions at the end of the 10-day period.

Curve IV. Per cent mortality during the 10-day period.

None of the rats, 33 days of age or older, failed to survive the 10-day experimental period.

Incidence of hemorrhagic degeneration in 40-gm. female rats. The results of choline deficiency were less severe in female rats than in male rats of the same age and weight. A longer experimental period was required for the production of the deficiency, and the degenerative enlargement of the kidneys

TABLE 3

The deposition of liver fat and the occurrence of hemorrhagic degeneration in 40-gm. female rats on a low choline diet

GROUP NO. AND DIET	1 AC32	2 AC32	3 AC32	4 AC33	5 AC33
Experimental period, days	6	10	15	6	10
No. of rats in group	15	22	15	10	21
Average final body weight, gm.	47	61	65	51	63
Average weight of liver, gm.	2.98	4.86	5.23	3.82	5.21
Average weight of liver fat, mg.	635	1288	1271	935	1690
Per cent of liver fat in liver	21.3	26.4	24.3	24.4	32.4
Average weight of kidneys (per pair), mg.	538	780	775	606	850
Kidney weight as per cent of normal kidney weight	95	112	107	99	119
Appearance of kidneys					
Normal, %	100	23	40	100	19
Recovered, %	0	23	46	0	14
Partially recovered, %	0	14	7	0	9
Hemorrhagic, %	0	38	7	0	58

was not as marked as in males. The results of these experiments on female rats, 38 to 42 gm. in weight and 24 to 26 days of age, are shown in table 3. There was no indication whatever of renal hemorrhage after a 6-day period on diet AC32 (group 1) or on diet AC33 (group 4). Severe hemorrhage occurred in twenty-seven out of twenty-nine young males after a 6-day period on diet AC32 (group 1, table 2). The characteristic results of the deficiency were observed after a 10-day period on these two diets (groups 2 and 5). All of the rats survived and recovery was nearly complete after a 15-day

period (group 3). The usual deposition of liver fat occurred in these animals.

Curative action of choline. The effect of the administration of choline after the appearance of renal lesions is shown in table 4. Four groups of male rats, 38 to 42 gm. in weight and 23 to 24 days of age were fed diet AC32. Groups 1, 2 and 3 (table 4) were killed after 5, 6, and 10 days respectively. On the sixth day the rats in group 4 were changed to diet AC32

TABLE 4

The effect of administration of choline after appearance of hemorrhagic degeneration in 40-gm. male rats on diet AC32

GROUP NO.	1	2	3	4[1]
Experimental period, days	5	6	10	10
No. of rats in group	30	29	30	33
Average final body weight, gm.	50	54	53	58
Average weight of liver, gm.	3.64	3.76	3.80	3.68
Average weight of liver fat, mg.	942	988	858	552
Average weight of kidneys (per pair), mg.	630	795	870	788
Kidney weight as per cent of normal kidney weight	105	124	140	117
Appearance of kidneys				
Normal, no. of rats	23	2	2	10
Recovered, no. of rats	0	0	5	17
Partially recovered, no. of rats	0	0	11	5
Hemorrhagic, no. of rats	7	27	1	0
No. of deaths	0	0	11	1

[1] Diet AC32 for 6 days. From sixth to tenth days diet AC32 supplemented with 3.0 mg. of choline chloride per gram of food. Two subcutaneous injections of 5 mg. of choline chloride on both sixth and seventh days.

supplemented with 3.0 mg. of choline chloride per gram of food. In addition each rat was given two subcutaneous injections of a solution of choline chloride (5 mg. per 0.25 cc.) on both the sixth and seventh days. Groups 1 and 2 illustrate the very rapid onset of the hemorrhagic condition. Only two out of twenty-nine rats failed to show the renal lesions on the sixth day. After 10 days eleven rats had succumbed to the toxic effects of the choline deficiency, the majority of the fatalities

having occurred on the seventh day (group 3). On the tenth day the hemorrhagic condition was present in twelve of the nineteen surviving rats in group 3 but in eleven of the twelve rats the recovery process had started as evidenced by the partial disappearance of blood from small areas on the cortex. Even though the recovery of surviving rats was rapid without added choline the administration of choline greatly accelerated the process. On the tenth day the rats in group 4, which had been given choline beginning with the sixth day, were definitely in better condition than the rats in group 3. Only one rat failed to respond to the administration of choline. This animal was nearly moribund on the sixth day and died on the seventh day. Of the thirty-two survivors only five showed remaining hemorrhagic areas and in every case these areas were small. This curative action of choline was also illustrated by the changes in the weight of the kidneys. It was assumed that this weight was approximately the same in groups 2, 3 and 4 on the sixth day. The weight continued to increase in the untreated rats (group 3) and decreased in the treated animals (group 4). The 4 days of choline administration resulted in the removal of some but not all of the extra fat deposited in the liver during the first 6 days.

The injection of choline during the acute stage of the renal degeneration was necessary in order to demonstrate its beneficial effect. It was found in preliminary experiments that the consumption of food on the sixth and seventh days, at which time the rats were acutely ill, was too low to supply sufficient choline if only 3 mg. were added per gram of food.

Protective action of choline. The observations on the relation of age and weight to the occurrence and severity of hemorrhagic degeneration indicated that young male rats, 20 days of age, were the most satisfactory animals for the demonstration of the result of choline deficiency and of the protective effect of choline and of choline-like substances. In such rats the incidence of the deficiency was high but the mortality was low. Furthermore, it was evident that more consistent results could be obtained by using a 7-day rather than a 10-day ex-

perimental period. The shorter period permitted the determination of the renal damage during the acute phase uncomplicated by partial recovery of the surviving rats. Table 5 shows the results obtained with male rats, 20 days of age and 24 to 30 gm. in weight, after a 7-day period on the experimental

TABLE 5

The effect of choline on occurrence and severity of hemorrhagic degeneration in 20-day-old male rats, during a 7-day experimental period

GROUP NO. AND DIET	1 AC34	2 AC34	3 AC34	4 AC34	5 AC32	6 AC32	7 AC32	8 AC32	9 AC32	10 AC32	11 AC32
No. of rats in group	21	11	19	13	30	11	10	11	10	10	10
Average initial body weight, gm.	28	26	27	27	28	28	26	27	27	28	28
Average final body weight, gm.	39	40	42	44	34	41	40	40	48	47	45
Choline chloride added per gm. of food, mg.	0	0.06	0.12	0.25	0	0.06	0.12	0.25	0.50	0.75	1.00
Average weight of liver, gm.	2.69	2.89	2.90	2.90	2.31	2.78	2.83	2.64	3.05	2.67	2.15
Liver weight as per cent of body weight [1]	6.83	7.24	6.95	6.64	6.80	6.78	7.07	6.60	6.35	5.68	4.77
Average weight of kidneys (per pair), mg.	678	591	544	517	709	772	707	577	526	533	505
Kidney weight as per cent of body weight [2]	1.72	1.48	1.31	1.16	2.08	1.88	1.76	1.49	1.10	1.13	1.12
Appearance of kidneys Normal, %	10	36	68	100	7	0	0	9	100	100	100
Moderately hemorrhagic, %	23	46	27	0	13	18	40	45	0	0	0
Severely hemorrhagic, %	67	18	5	0	80	82	60	46	0	0	0

[1] Normal per cent of body weight for rats in this colony, 4.31.
[2] Normal per cent of body weight for rats in this colony, 1.1 to 1.2.

diet. The two diets used, AC34 and AC32, were identical except for the addition of 0.3% of cystine to the latter. Although the weights of the livers were included in the results shown in table 5, this measure of the fatty liver effect appeared of far less value in estimating the severity of the deficiency than the weight and appearance of the kidneys. The latter were found to be exceedingly sensitive indicators of the absence of

choline in the diet not only because renal hemorrhage occurred as a sign of the deficiency but also because the resulting renal enlargement varied with the extent of the deficiency. The presence of even slightly hemorrhagic areas in the cortex was readily recognizable by direct observation.

The weight of the kidneys of young normal rats was 1.0 to 1.2% of the body weight. After the onset of hemorrhagic degeneration this value was increased to 1.72% on the basal diet (group 1) and to 2.08% on the basal diet plus 0.3% of cystine (group 5). Groups 2 to 4 and 6 to 11 show the correlation between the weight and appearance of the kidneys and the addition of choline to these two diets. These results indicated that, in the range of suboptimal levels of choline, there was almost a straight line relationship between the choline supplement and the kidney weight, expressed as per cent of body weight. A study is being made of the possibility of using this response of the kidneys to a lack of choline for the assay of this and related substances having a choline-like action or an opposite effect. This latter effect is illustrated in table 5 in the comparison of the protective levels of choline on the basal diet with and without added cystine (groups 4 and 9).

The severity of the renal pathology observed in this investigation emphasized the necessity of additions of choline to such experimental diets. The food consumption of the rats on diet AC34 was 3 to 4 gm. per day so that approximately 1 mg. of choline chloride was required daily to prevent the renal lesions (group 4, table 5). Twice as much was necessary if 0.3% of cystine was included in the ration (group 9). Previous experiments have indicated that the choline requirement for the prevention of fatty livers is two to three times that for the prevention of hemorrhagic degeneration.

Diet AC34 was similar to many which have been used in recent years in investigations in which the young rat has been the experimental animal. It is possible that the deficiency has occurred previously and escaped detection because the rats did not happen to have been sacrificed during the acute stage. Rats which have survived a moderately hemorrhagic condition show normal early growth curves except for the temporary

inhibition of growth between the fifth and tenth days. The possible effects of this unrecognized pathological state on the outcome of such investigations remain to be determined.

SUMMARY

1. Hemorrhagic degeneration occurred within 10 days in male rats, 20 to 30 days of age, on a low choline diet containing 0.3% of added cystine.

2. A marked decrease in the incidence of the deficiency in similar rats, 33 days of age or older, suggested that there is a corresponding decrease in the choline requirement of rats over 30 days of age.

3. The renal lesions in 40-gm. male rats, 21 to 26 days of age, were most severe on the sixth and seventh days. Only 60 to 80% of these rats survived the 10-day period.

4. The results of choline deficiency appeared more slowly and were less severe in young female rats than in male rats of the same age and weight.

5. The administration of choline after the appearance of hemorrhagic degeneration accelerated the recovery from the acute stage of the deficiency.

6. The increase in weight of the kidneys of male rats, 20 days of age and 24 to 30 gm. in weight, after a 7-day period on diets containing suboptimal levels of added choline was proportional to the severity of the deficiency of choline.

The writer wishes to thank W. J. Bailey and D. J. Mulford for their assistance in part of this investigation.

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WHEAT AS A DIETARY SOURCE OF IRON

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ONE FIGURE

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It was shown by the classic investigations of Sherman ('32) and it has been emphasized by the work of Rose ('38) that wheat and other cereals are important dietary sources of iron, particularly for people of the so-called low income group. It is well known that refined milling products, so largely used in the diet, contain less iron than the entire grain. The present noteworthy tendency to restore important nutritional essentials to processed foods demands that accurate information about the composition and nutritive value of the unprocessed foods be available. Information about wheat as a dietary source of iron has been conflicting.

The results of a number of published reports on the average iron content of different varieties of wheat are included in the data provided by table 1. Sherman considered that the average iron content of whole wheat is about 5 mg. per 100 gm. Many of the more recent reports indicate that the iron content of wheat is usually between 3 and 4 mg. per each 100 gm. Some variation is to be expected in natural food products but a variation of from 1 to 32 mg. per 100 gm. in wheat as reported by Greaves and Hirst ('29) seems extreme.

The nutritional value of the iron of wheat has been studied by assays with anemic rats and by chemical analyses of so-called available iron. From the rate of increase in the con-

centration of hemoglobin following the feeding of wheat to anemic rats Elvehjem, Hart and Sherman ('33) concluded that the iron of wheat was about half as effective as an inorganic salt of iron. Others have found by similar feeding tests that the iron of wheat is as well utilized as inorganic iron salts (Vahlteich, Rose and MacLeod, '36; Smith and Otis, '37). The chemical method (Hill, '30; Elvehjem, Hart and Sherman, '33) for estimating the nutritional value of the iron of foods also has given conflicting results. The fraction of the total iron which, after treatment with a reducing agent, reacts with dipyridyl has been variously referred to as available, inorganic and ionizable iron. Hahn and Whipple ('38) have criticized the use of the term "available iron" because it is not at once apparent from this expression that 100% availability, for example, means only that the iron of the food is utilized by the body as well as an equal amount of iron in the form of an inorganic salt. "Inorganic iron" and "ionizable iron" are terms which may be criticized on chemical grounds. We believe that a term to be preferred for the iron of foods and tissues which reacts with dipyridyl when treated according to Hill's method, or modifications thereof, is "ionogenic iron." This term, which may be defined according to Shohl's terminology ('39) as the iron which yields ions in the course of body processes, has been adopted in the present report. The values for the ionogenic iron content of wheat included in table 1 emphasize the conflicting nature of the data obtained by chemical methods for the estimation of the nutritive value of the iron of foods. It is not clear from these data whether the iron of wheat is 43 or 92% as efficient as an equal amount of iron in the form of ferric chloride.

The present report provides some additional data on the total and ionogenic iron content on a number of varieties of American grown wheat. Bioassays of the availability of the iron of two samples of wheat are also reported. The data from both studies are discussed briefly with reference to published evidence in the literature.

TABLE 1

Survey of published reports of the average iron content of wheat

REFERENCE	TOTAL FE	IONOGENIC FE	IONOGENIC FE
	mg./100 gm.	*mg./100 gm.*	*%*
Leach ('09)	3.06		
Sherman ('32)	5.00		
Peterson and Elvehjem ('28)	3.72		
Rose and Vahlteich ('32)	3.30		
Elvehjem, Hart and Sherman ('33)	5.00	2.33	47
Sullivan ('33)	3.10*		
Rose, Vahlteich and MacLeod ('34)	3.16		
Saiki et al. ('34)	3.5		
Vahlteich, Rose and MacLeod ('36)	3.33		
Smith and Otis ('37)	5.18	4.77	92
Aykroyd ('37)	5.37		
Goswami and Basu ('38)	5.5	2.74	50
Ranganathan ('38)	3.97	1.69	43
Free and Bing (present study)	3.94	3.19	81

* Dry basis.

EXPERIMENTAL

Sources of wheat. Under the Official Grain Standards Act of the United States, wheat is divided into five separate classes. These differ in habits of growth, in appearance and in chemical composition and physical properties. We are indebted to Dr. R. M. Bethke of the Ohio Agricultural Experiment Station, Wooster, for three samples of soft red winter wheat (nos. 1, 2 and 3 in table 2) and to the late Dr. Kurt S. Franke of the South Dakota Agricultural Experiment Station, Brookings, for the samples of hard red spring wheat, hard red winter wheat, and durum wheat (nos. 7 to 11 inclusive in table 2). The remaining samples were obtained from local stores (nos. 4 and 5 in table 2) and from the Miami Milling Co., Oxford, Ohio (no. 6 in table 2), and were not identified as to variety, although they belonged to the class of soft red winter wheat. Thus four of the five classes of wheat were included in the present study and, according to Hughes and Henson ('30), the one class not included, soft white wheat, is not grown to any large extent.

Chemical methods. To determine the total iron content a sample of 10 gm. was weighed into a porcelain dish, dried and 0.5 gm. of iron-free calcium acetate added. The sample was

ashed in an electric muffle furnace at from 500° to 550°C., after which the iron was determined by the thioglycolic acid colorimetric method of Hanzal ('33), using the technic described by Bing, Saurwein and Myers ('34).

The determinations of the ionogenic iron content of wheat were carried out on samples which had been finely ground in

TABLE 2

Total and ionogenic iron of wheat

VARIETY AND CLASS	MOISTURE	TOTAL IRON	IONOGENIC IRON	IONOGENIC IRON
	%	*mg. per 100 gm.*	*mg. per 100 gm.*	*% of total iron*
1. Nabob Soft red winter wheat	8.3	2.90	2.46	85
2. Fulhio Soft red winter wheat	9.6	3.90	3.19	82
3. Trumbull Soft red winter wheat	8.2	3.48	2.76	79
4. Unknown (no. 1) Soft red winter wheat	10.0	4.87	4.04	83
5. Unknown (no. 2) Soft red winter wheat	11.1	3.72	3.23	87
6. Unknown (no. 3) Soft red winter wheat	10.3	3.04	2.60	86
7. Turkey Hard red winter wheat	7.5	4.20	3.14	75
8. Ceres Hard red spring wheat	7.3	3.66	2.95	81
9. Marquis Hard red spring wheat	7.5	4.36	3.18	73
10. Acme Durum wheat	7.9	4.40	3.85	88
11. Mindum Durum wheat	6.6	4.81	3.72	77

a porcelain mortar. The method used was similar to that described by Elvehjem, Hart and Sherman ('33). Because certain modifications have been employed and because of the variation in results obtained by different investigators, the exact technic is described briefly.

A sample of finely ground wheat weighing 3.000 gm. is placed in a round-bottom centrifuge tube, capacity 50 cc. To

the tube are added 5 cc. of M sodium acetate, 2 cc. of 10% acetic acid, and 5 cc. of water, which provides a buffer mixture with a pH about 5.0 for the extraction of the iron. To reduce the iron to the ferrous condition, 0.25 gm. of iron-free sodium hydrosulfite is added. This is followed by a small quantity, about 1 mg., of dipyridyl, which develops a red color with ferrous iron. The contents of the tube are mixed, the tube is stoppered and allowed to stand at room temperature for from 36 to 48 hours with occasional stirring. The longer extraction time is necessary with samples of hard wheat. At the end of this time 5 cc. of 95% ethyl alcohol are added to precipitate soluble proteins and other extracted substances. The tube is then placed in a refrigerator at 5°C. for 12 hours, by which means turbidity of the final solution is avoided. The material is then centrifuged while cold and an aliquot portion of the clear red extract is removed with a pipette.

The amount of iron is determined by colorimetric comparison, using a series of standards in calibrated test tubes supplied with paraffined cork stoppers. The readings are made in a comparator block in daylight, with a white porcelain plate as a background. It is convenient to select an amount of solution so that the readings are made with concentrations of from 0.13 to 0.16 mg. of iron per 100 cc.

The standards are prepared from a solution of 0.0001 M iron dipyridyl in 0.2 M sodium acetate-acetic acid buffer, with an excess of dipyridyl and sodium hydrosulfite. A series of tubes providing a range of from 0.070 to 0.300 mg. of iron per 100 cc. was made. In all determinations a blank was run to allow for the yellow colored material extracted from the wheat. The blank is prepared by weighing out 3.000 gm. of the sample and treating this exactly the same as the sample being analyzed, except that no dipyridyl is added. This control tube is placed behind the standard tube and a tube of water is placed behind the "unknown" tube when colorimetric comparisons are made.

Recovery experiments with added iron gave theoretical values within $\pm 5\%$. Longer extraction did not increase the

amount of ionogenic iron obtained from any of the wheat samples. In order to ascertain whether prolonged extraction might have a destructive effect on organic iron compounds, hematin was added to samples of wheat. Although the amount of iron in the form of hematin which was added was equal to twice the iron content of the wheat, there was no change in the apparent ionogenic iron as determined by the present method.

The results of the determinations of total and ionogenic iron in eleven specimens of wheat as determined by the methods described are provided in table 2. This table shows that the total iron varied from 2.90 to 4.87 mg. per 100 gm. of wheat and the ionogenic iron from 2.46 to 4.04 mg. per 100 gm. of wheat. The averages for all samples, regardless of variety, were 3.94 mg. of total iron and 3.19 mg. of ionogenic iron per 100 gm. of wheat. The variations in iron content appear to be unrelated to the variety of the wheat. The ionogenic iron expressed as a percentage of the total iron varied from 73 to 88%, and the average value was 81%. The ionogenic iron appears to be a relatively constant proportion of the total iron. As a result, there were some samples having a high total iron content, in which the ionogenic iron was considerably higher than the total iron of samples of wheat having a relatively low iron content.

The nature of the organic iron of wheat has not been determined. Wheat gives a positive test with benzidine and hydrogen peroxide which may indicate the presence of a hematin compound. The possibility that even most of the residual iron is present in some simple inorganic combination which is not extracted by the technic employed cannot be denied.

The results of the present study are not in agreement with those of Elvehjem, Hart and Sherman ('33) or of two investigators in India (Goswami and Basu, '38; Ranganathan, '38) who found that only about one-half of the iron in wheat is available by chemical tests. Our results are somewhat lower than those of Smith and Otis ('37), who found by the dipyridyl method that 92% of the total iron of wheat is ionogenic. When

Myers, Remp and Bing ('35) first tested the "availability" of iron in bread by the dipyridyl method they found only about 50% of the total iron to be ionogenic. Later studies showed that with a longer period of extraction, from 94 to 97% of the total iron in white, rye and whole wheat bread is ionogenic (Remp, '35). McCance ('39) likewise has called attention to discrepancies in the values recorded for the ionogenic iron content of foods by different workers.

Biological assay. Although the chemical method for the estimation of the nutritional value of the iron of foods may become acceptable, there is no doubt that the figures obtained at the present time not only are apt to vary with different technics but also the results are difficult to interpret. The bioassay of the iron of foods is tedious and time-consuming, but it remains the best method for the purpose of determining whether the iron in a food product can be utilized by the body. Rose and Vahlteich ('32) have shown by feeding experiments with anemic rats that the iron of wheat is as well utilized as the iron of ferric chloride. Smith and Otis ('37) likewise have reported that the iron of wheat is well utilized. Elvehjem, Hart and Sherman ('33), however, reported that, as a source of iron, wheat is about half as valuable as iron in the form of a simple inorganic salt. Vahlteich, Rose and MacLeod ('36) have presented evidence indicating that the iron of wheat which has been treated with diastase is even better utilized than an equal amount of iron in the form of an iron salt. Remp ('35) also has found that when wheat is fed in the form of bread, the iron is somewhat better utilized than an equal amount of iron in the form of ferric chloride.

In the present experiments assays were made of the iron value of two varieties of soft red winter wheat. Briefly, the technic was as follows: Albino rats were made anemic by restriction to a milk diet from the time of weaning. Raw Guernsey milk obtained by milking directly in glass bottles was used. When the hemoglobin level of the blood was below 4.0 gm. per 100 cc., the animals were placed on the experimental diets. One group of five animals served as controls and each

rat in this group was given daily 0.25 mg. of iron in the form of ferric chloride. A group of six animals received sufficient Trumbull wheat (a soft red winter wheat) to supply daily 0.25 mg. of iron to each rat. Another group of six animals received Nabob wheat (a soft red winter wheat) in quantities sufficient to provide 0.25 mg. of iron per day. No difficulty was experienced in securing complete consumption of the amounts of wheat necessary to provide the selected dosage of iron. The rats in all three groups were given daily supplements of 0.05 mg. of copper as copper sulphate and 0.05 mg. of manganese as manganous chloride. All animals received as much milk as they would drink, following the consumption of the daily wheat ration. The food intake of each animal was measured and in

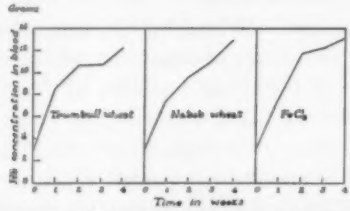

Fig. 1 The average hemoglobin concentration, as grams per 100 cc. of blood, is shown for anemic rats which received daily 0.25 mg. of Fe as wheat or $FeCl_3$.

that way the intake of iron was calculated. The animals grew well for the duration of the experiment and there was no particular difference in the response of male and female animals. Each group contained, whenever possible, equal numbers of males and females. An examination of other data obtained in our laboratory shows that if there is any difference in the response of males and females it is very small, even for animals receiving doses of iron smaller than 0.25 mg. daily. Perhaps this is because no difference in the food consumption of males and females was observed during the short time of the feeding test.

The hemoglobin concentration of the blood was determined weekly during the 4-week period. The average values for the three groups of animals are plotted in figure 1. It may be

mentioned that the response of the animals receiving the ferric chloride was similar to that of many other animals in our colony which had received similar treatment. The average increase of hemoglobin in the rats receiving ferric chloride was 10.1 gm. per 100 cc. of blood. The rats receiving Nabob wheat showed an increase of 10.0 gm. and the rats receiving Trumbull wheat 9.1 gm. of hemoglobin per 100 cc. of blood. The increase in hemoglobin in the three groups of animals is considered to be essentially the same.

Although the response in hemoglobin concentration is usually taken as a measure of the availability of iron, we have found it desirable to supplement the data by analysis of the animals for iron content. A discussion of the methods of assay for iron need not be made in the present report. Briefly the method is as follows: At the end of the experiment, in this case at the end of 4 weeks, the animals are killed by prolonged anesthesia with ether. The carcasses are then cleaned, washed with distilled water, and placed in the refrigerator for several hours. The stomach and intestines are then dissected and removed and the rest of the carcass is ashed in an electric muffle furnace. The method of analysis has already been described (Bing, Saurwein and Myers, '34).

The retention of iron is determined by subtracting from the iron content of the body the amount estimated to have been present at the beginning of the experiment. The initial iron content is calculated from analyses of the bodies of control animals having a similar nutritional history, the same weight and hemoglobin concentration in the blood. In table 3 are shown the results of this method of determining the retention of the iron. The calculated initial content of iron was on the average in each group 1 mg., or a little more, and after receiving the indicated treatment for 4 weeks the iron content had increased by from 3.3 to 3.4 mg. The percentage utilization is determined by multiplying the value for the retention of iron by 100 and dividing by the estimated iron intake during the experimental period. As shown in the table, there was a slight variation in the total iron intake, depending on spillage

of food, consumption of milk, and such factors, all of which were taken into account. The percentage utilization of the iron of ferric chloride under the conditions of the experiments was 47% of the intake. In the wheat 48% of the iron of one sample was retained and 51% of the iron of the other sample. These data show clearly that the iron of wheat is just as well utilized as an equal amount of iron in the form of ferric chloride.

TABLE 3

Results of biological assay of the availability of the iron of wheat

SOURCE OF IRON	TOTAL IRON INTAKE	CALCULATED INITIAL BODY IRON	FINAL BODY IRON	INCREASE IN IRON	RETENTION
	mg.	*mg.*	*mg.*	*mg.*	%
Ferric chloride (average of 5 rats)	7.29	1.00	4.39	3.39	47
Trumbull wheat (average of 6 rats)	7.01	1.10	4.43	3.33	48
Nabob wheat (average of 6 rats)	6.80	1.03	4.48	3.45	51

SUMMARY AND CONCLUSIONS

The iron content of eleven samples of American grown wheat varied from 2.90 to 4.87 mg. per 100 gm., and the average was 3.94 mg. These values are in accord with most of the recent reports of the iron content of wheat, which show that about 3 to 4 mg. of iron are present per 100 gm. It was shown by the dipyridyl method that an average of 81% of the total iron of wheat is ionogenic iron (also called available and inorganic iron). The proportion of the total iron in the form of ionogenic iron in the present series of determinations ranged from 73 to 88%. Within these limits the ionogenic iron is a constant proportion of the total iron. By means of biological assays with anemic rats it was shown that the iron of wheat is fully as available to the organism as an equal amount of iron in the form of ferric chloride. Under the conditions described, about one-half of the iron of ferric chloride or of finely ground whole wheat is retained by the anemic rat. In view of the results of

the feeding tests it is difficult to interpret the values obtained with the chemical method for the estimation of ionogenic iron.

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EFFECTS OF SIMPLE DIETARY ALTERATIONS UPON RETENTION OF POSITIVE AND NEGATIVE MINERALS BY CHILDREN[1]

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The rapidly developing science of nutrition has demanded specific knowledge concerning the physiologic effects of individual foods as they undergo the various phases of digestion, absorption, and utilization. Frequently, in dietetic practice, one food is substituted for another on the basis of a single food principle without regard for equally important component parts of the diet. It is essential that consideration be given to the physiologic events consequent to the changed characteristics of the mixed diet as a result of altering the quantity of only one food, which in itself is subject to wide variations in composition. Isocaloric diets and the total nitrogen of diets have been studied, but little is known of the minerals in diets beyond some effects of different levels and proportions of calcium and phosphorus upon metabolism.

No two natural foods are exactly alike; their caloric values may be identical while their carbohydrates vary in availability and the indigestible portions possess different physical characteristics. Likewise, with similar calorigenic qualities their mineral contents may be unlike and the various minerals present in dissimilar proportions; the total calorie and nitrogen

[1] The expenses of this study were contributed in part by the United Fruit Company, Boston, through the courtesy of Mr. P. K. Reynolds.

contents may be similar but the various vitamins may not be present in effective proportions; they may have the same total nitrogen content while the proportions of the different amino acids in the proteins are so different that one food produces nutritive success and another failure. Positive knowledge of the physiologic effects produced by dietary alterations is needed. The nutritive value of these effects must be determined if dietetic requirements are to be adequately provided for during growth of the child and maintenance of the adult, in both health and disease.

The present investigation, only one phase of an extensive study of growth, deals with the effects of simple dietary alterations upon retention of positive and negative minerals by normal, active children. The intakes of positive and negative minerals were maintained at approximately constant levels for each subject but the distribution of component inorganic elements within the positive (calcium, magnesium, sodium and potassium) and negative (phosphorus, sulfur and chlorine) mineral groups were varied during the experimental periods by altering the proportions of banana (an alkaline-ash food with a high concentration of potassium), apple, and cereal in adequate mixed diets. During both the pre-experimental and experimental periods the alkaline-ash values were sufficient for growth needs (Shohl, '23) although the values were approximately doubled during the latter.[2]

METHODS

Nine children, 5 to 8 years of age (four girls and five boys), who through intensive physiologic and medical observation were known to be in favorable nutritive condition, were the subjects of metabolic study during pre-experimental and ex-

[2] The recent trend toward greater accuracy in referring to electropositive and electronegative elements or radicals has prompted the use of terms based upon the nomenclature used by Shohl ('39) in his monograph on "Mineral Metabolism." Former use of the words "base" and "acid" has been replaced by "positive minerals, or cations" and "negative minerals or anions." "Ion balance" or "excess ions" has replaced acid-base balance for urine, feces, and retentions and alkaline-ash value is used for the "excess of positive minerals" in intakes.

perimental periods ranging from 20 to 55 consecutive days for each child, a total of 640 days. The Methodist Children's Village afforded ideal facilities for metabolic observations. The children lived together in a home unit with a housemother; went to school and to Sunday school in the Village; enjoyed their customary freedom of play out of doors with pets and playthings; and were given every opportunity for unhampered social and physical development. They slept in individual beds in pleasant airy rooms, and were at all times, even going to and from school, under the continuous observation of a trained person, so it is known that no metabolic samples were lost or extra foods eaten. Three young women, trained in nutrition and dietetics, quantitatively prepared and served the food and collected all the metabolic specimens which were analyzed [3] by the staff of the Research Laboratory of the Children's Fund of Michigan. Two housemothers gave special attention to the emotional care and management of the children.

The children's diets were composed of twenty-one common foods [4] and conformed to accepted dietetic practice, providing averages of approximately 0.8 gm. of calcium and 60 gm. of protein per child per day, in addition to ample amounts of vitamins. They were comparable qualitatively but quantity was adjusted to meet the requirements of the individual's age, size and activity. Each subject served as his own control, for growth and response to a given dietary regimen differ among children, owing to dissimilar inheritance and treatment during growth and development. Each child's gastrointestinal pattern (Macy, Reynolds, Bates and Souders, '40) and response to different types of test meals were known (Reynolds, Macy and

[3] The food, urine, and feces were each analyzed for nitrogen, calcium, magnesium, sodium, potassium, phosphorus, sulfur, and chlorine. The chemical methods, omitted by space requirements, may be obtained from the authors. Methods of analysis will be presented in detail in a forthcoming monograph upon the chemical growth of children.

[4] The mixed diet contained the following foods: apple, banana, lean beef, white and whole wheat bread, shredded wheat, cabbage, carrots, American cheese, eggs, lettuce, milk, orange juice, peanut butter, potato, spinach, tomato juice, graham crackers, butter, sugar and salt.

Bates, '40). The health status,[5] care, and management of the children, procedures, and chemical methods are detailed elsewhere (Hunscher, Hummel and Macy, '39; Hummel, Hunscher and Macy, '39).

Banana was selected as the basis of the dietary manipulations because the fruit is generally liked (Alvarez and Hinshaw, '35) and has been proved beneficial to well infants (Johnston, '27; von Meysenbug, '27), those with debilitated digestive disorders (Haas, '32; Brubaker, '37) and scurvy (Johnston, '27) and the development and well-being of children (Brown and Courtney, '29; Roberts, Blair, Austin and Steininger, '39). In addition, the ash of banana is high in potassium, low in calcium, and, when 100 gm. were added or substituted for cereal in the daily mixed diet, its high alkaline-ash value permitted an increase of approximately 50% in the alkaline-ash values of the intakes per kilogram of body weight without altering the total minerals content, even though the pre-experimental mixed diets contained different amounts of apple and banana. Seven children were already receiving both apple and banana in their mixed diets, eliminating the possibility of effects from intolerance or allergy in the results.

The alterations made in the diets of eight subjects during the experimental period consisted of the addition or substitution for cereal of 100 gm. of banana. One subject had other additions (10 gm. white bread; 20 gm. cereal) to the diet during the experimental period. Five of the eight children were receiving identical amounts of raw apple and raw banana combined throughout the pre-experimental and experimental periods, 200 gm. daily during the pre-experimental period and 300 gm. during the experimental period. However, three children received 100 and two subjects 200 gm. of apple daily during the pre-experimental period; the former receiving 100 gm. of banana per day and the latter receiving no banana. This variation in the mixed diets of the five children produced a difference between the proportionate compositions of the

[5] Marsh W. Poole, M.D., graciously made the medical examinations and physical measurements and gave special attention to the health of the children.

diets which was accentuated during the experimental period even though the changes in dietary were the same.

RESULTS AND DISCUSSION

The effect of each variation upon the mineral composition of the food intakes is shown in table 1. It is important to note that the addition or substitution of 100 gm. of banana produced an increase of approximately 7.7 meq. in the alkaline-ash value of the intakes regardless of other differences in the diets. When added to the mixed diet, 100 gm. of banana increased the intake of total minerals 18.1 meq.; when substituted for cereal the intake was only increased by 2.0 meq. In relation to the quantities of total minerals in the mixed diets these changes are small as is the difference in total minerals between 100 gm. of banana and 100 gm. of apple (12.9 meq.). Per kilogram of body weight these changes become even less significant, although the conservative changes did cause significant and wide variations in the proportions of the different individual elements in the mixed diets (Hunscher, Hummel and Macy, '40). These variations in individual elements, significant in themselves, did not produce significant differences in the intakes of total positive or total negative minerals per kilogram of body weight (table 2). During the pre-experimental period the subjects' intakes (table 2) provided an average daily excess of 0.67 meq. of positive minerals per kilogram of body weight. This was increased 0.43 meq. (0.67 to 1.10 meq.) per kilogram of body weight during the experimental period, making the average mineral intakes 1.10 meq. per kilogram of body weight. These amounts are comparable with the excess of 10 cc. of 0.1 *N* base (1.0 meq.) which Shohl ('23) considers necessary for growth.

Banana and apple contain about 19 and 12% available carbohydrate, respectively (Widdowson and McCance, '35), and have other beneficial dietary properties that are not fully understood (Bergeim, Hanszen and Arnold, '36; Baumann and Forschner-Böke, '34; von Loesecke, '30). Apple and banana contain similar amounts of "unavailable" carbohy-

TABLE 1

Average daily chemical variations in diets consequent to variations in their banana, apple and cereal contents

	PRE-EXPERIMENTAL PERIOD MIXED DIET [1] (Group I)	ADDITION OF 100 GM. BANANA (All groups)	DELETION OF CEREAL [2] (Groups II, III)	CHANGES [3] DUE TO SUBSTITUTION (Groups II, III)	CHANGES IN ADDITION TO BANANA (Group IV)	VARIATION DUE TO 100 GM. APPLE (Groups II, III)
Total calories	1900	99	93	+6	255	63
Nitrogen (gm.)	10.3	0.1	0.4	—0.3	0.9	0.1
Protein (gm.)	64.5	0.7	2.3	—1.6	5.4	0.4
Fat (gm.)	69.5	0.1	0.6	—0.5	15.4	0.2
Total minerals (meq.)	419.2	18.1	16.1	+2.0	41.8	5.2
Positive minerals (meq.)	217.3	12.9	8.1	+4.8	21.5	4.1
Calcium (meq.)	38.3	0.2	0.7	—0.5	2.2	0.2
Magnesium (meq.)	21.9	2.7	0.8	+1.9	1.5	0.6
Sodium (meq.)	94.6	0.2	5.1	—4.9	13.0	0.1
Potassium (meq.)	62.5	9.8	1.5	+8.3	4.8	3.2
Negative minerals (meq.)	201.9	5.2	8.0	—2.8	20.3	1.1
Phosphorus (meq.)	62.5	1.6	3.1	—1.5	4.6	0.8
Chlorine (meq.)	96.2	3.2	3.0	+0.2	10.7	0.1
Sulfur (meq.)	43.2	0.4	1.9	—1.5	5.0	0.2
Alkaline-ash value (meq.)	15.4	7.7	0.1	+7.6	1.2	3.0

[1] The caloric value of the diets varied with intakes of sugar and diet levels were adjusted to age, size, and activity of the individuals. The diet value given is for D.P. All data are from analyses.

[2] Ten grams of white bread and 20 gm. of shredded wheat or corn flakes.

[3] Change in content when 100 gm. of banana were substituted for cereal.

drate but their proportionate compositions of lignin, cellulose and hemicellulose are dissimilar. These variations and their influence upon the disappearance of complex carbohydrates from the tracts of these children have been discussed (Hummel, Shepherd and Macy, '39; Shepherd, Hummel and Macy, '40). It is known that banana and apple influence the bactericidal power of the stomach (Hanszen, '34) and that pectin (Manville, Bradway and McMinis, '36; Ziegelmayer, '36) and the flora of the intestine (von Meysenbug and Fine, '36) influence nutritional processes. Myers and Rose ('17) found the reducing sugar in the very green, medium ripe, and very ripe banana to be 0.55, 2.88 and 7.08%; the non-reducing sugar (sucrose), 1.20, 12.52 and 11.10%; and the total sugar, 1.75, 15.40 and 18.18%, respectively. The bananas used in this investigation were fed when in a state of optimum [6] ripeness, and the apples were of the same variety and all purchased from the same source.

Ages, recumbent lengths, weights, the average daily caloric intakes, and nitrogen and mineral ion balances per kilogram of body weight for the nine children are presented in table 2. The average intakes per kilogram of body weight for the nine children show, for the experimental period, a slight increase in caloric value (2 cal.) and a reduction in nitrogen (10 mg.). In order to attain proportionate growth and development with a satisfactory distribution of the essential chemical elements in the skeletal, muscular, glandular and neural tissues, it is of synergistic nutritive advantage to have a continual storage of both positive and negative minerals, with a preponderance of the former (Shohl, '39; Hunscher, Hummel and Macy, '39). The subjects stored, on the average, 0.70 meq. of cations and 0.57 meq. of anions, a total of 1.27 meq. of minerals daily per kilogram of body weight during the pre-experimental period, the quantities increasing during the experimental period 0.20, 0.29 and 0.49 meq., respectively, which shows that, subject to individual variation, tissue synthesis was occurring through-

[6] Carefully selected bananas were furnished by an experienced wholesaler, Mr. B. E. Reiff, Fruit Dispatch Company, Detroit.

TABLE 2

Average daily balances of nitrogen, and positive and

		OBSERVATION PERIOD	INITIAL AGE	RECUMBENT LENGTH	WEIGHT	DIETARY CHANGES [1] Raw apple	DIETARY CHANGES [1] Raw banana	TOTAL ENERGY INTAKE
		days	*mo./days*	*cm.*	*kg.*	*gm./day*	*gm./day*	*Cal./kg.*
Group I:								
H.H.	Pre-experimental	35	84/24	119.8	20.76	100	100	90
	Experimental	40	85/28	120.2	21.33	100	200	93
F.C.	Pre-experimental	35	79/ 5	119.4	21.60	100	100	87
	Experimental	40	80/ 9	120.1	22.40	100	200	88
D.P.	Pre-experimental	35	98/20	136.0	27.68	100	100	69
	Experimental	30	99/24	137.1	27.60	100	200	72
Group II:								
R.S.	Pre-experimental	30	65/17	114.0	18.26	100	100	90
	Experimental	20	66/21	114.7	18.40	100	200	89
B.M.[5]	Pre-experimental	30	63/ 7	104.1	16.23	100	100	100
	Experimental	30	64/ 6	105.3	16.88	100	200	96
J.H.	Pre-experimental	30	55/22	106.9	16.84	100	100	97
	Experimental	30	56/21	107.4	16.87	100	200	97
Group III:								
J.M.[5]	Pre-experimental	40	75/ 3	116.4	20.64	200	—	85
	Experimental	45	76/17	117.4	21.58	200	100	82
B.F.[5]	Pre-experimental	40	69/12	110.5	18.43	200	—	95
	Experimental	50	70/21	111.4	19.28	200	100	91
Group IV:								
P.W.[5]	Pre-experimental	55	75/29	116.8	20.21	100	100	80
	Experimental	25	78/ 3	118.2	21.15	100	200	93
Pre-experimental period of		330 days:					Mean	87
							$\pm SE_m$[6]	
Experimental period of		310 days:					Mean	89
							$\pm SE_m$[6]	

[1] For groups II and III the 100 gm. banana added to the mixed diet during the experimental period was a substitution for 10 gm. white bread and 20 gm. cereal (shredded wheat or corn flakes) removed from the dietary. For group IV in addition to 100 gm. banana, 30 gm. potato, 10 gm. butter, and 50 gm. white bread were added to the daily diet during the experimental period.

[5] Sum of values for calcium, magnesium, sodium, potassium, phosphorus, chlorine and sulfur.

[6] Valences of 1.8 for phosphorus and 2 for sulfur have been used in calculations.

TABLE 2

egative minerals in response to altered diets

NITROGEN		MINERAL ASH-VALUES OF INTAKES				ION BALANCES IN EXCRETION [4]		RETENTION OF MINERAL IONS			
Intake	Retention	Total [2]	Positive	Negative [3]	Alkaline ash values	Urine	Feces	Total [2]	Cations	Anions [3]	Excess ions [4]
mg./kg.	*mg./kg.*	*meq./kg.*	*meq./kg.*	*meq./kg.*	*meq./kg.*	*meq./kg.*	*meq./kg.*	*meq./kg.*	*meq./kg.*	*meq./kg.*	*meq./kg.*
497	4	20.19	10.46	9.73	0.73	0.62⁻	1.21⁺	1.14	0.64	0.50	0.14⁺
489	20	20.50	10.79	9.71	1.08	0.04⁺	1.24⁺	1.72	0.76	0.96	0.20⁻
478	4	19.41	10.06	9.35	0.71	0.67⁻	1.25⁺	1.18	0.66	0.52	0.13⁺
466	18	19.51	10.27	9.24	1.03	0.02⁺	1.31⁺	1.86	0.78	1.08	0.30⁻
373	2	15.14	7.85	7.29	0.55	0.48⁻	0.84⁺	0.81	0.50	0.31	0.19⁺
378	2	15.84	8.34	7.50	0.84	0.39⁻	1.06⁺	1.61	0.89	0.72	0.16⁺
517	43	20.65	10.71	9.94	0.77	0.89⁻	1.36⁺	1.52	0.91	0.61	0.30⁺
503	19	20.47	10.97	9.50	1.47	0.61⁻	1.68⁺	1.00	0.70	0.30	0.40⁺
582	27	23.23	12.05	11.18	0.87	0.72⁻	1.49⁺	1.64	0.87	0.77	0.10⁺
549	26	22.32	11.96	10.36	1.60	0.36⁻	1.75⁺	1.87	1.04	0.83	0.21⁺
561	13	22.39	11.61	10.78	0.84	0.75⁻	1.33⁺	1.16	0.71	0.45	0.26⁺
549	10	22.34	11.97	10.37	1.60	0.38⁻	1.28⁺	1.82	1.26	0.56	0.70⁺
498	24	19.59	10.03	9.56	0.47	0.50⁻	1.15⁺	1.44	0.63	0.81	0.18⁻
464	29	18.82	9.81	9.01	0.80	0.26⁻	1.08⁺	1.72	0.85	0.87	0.02⁻
558	20	21.94	11.23	10.71	0.52	0.83⁻	1.42⁺	1.75	0.84	0.91	0.07⁻
520	36	21.07	10.98	10.09	0.89	0.42⁻	1.34⁺	1.81	0.89	0.92	0.03⁻
468	—6	18.66	9.68	8.98	0.70	0.67⁻	1.11⁺	0.94	0.60	0.34	0.26⁺
495	38	20.77	10.93	9.84	1.10	0.12⁺	1.23⁺	2.15	0.95	1.20	0.25⁻
500	13	19.98	10.33	9.65	0.67	0.68⁻	1.23⁺	1.27	0.70	0.57	0.12+
±7	±3	±0.27	±0.14	±0.14	±0.02	±0.04	±0.04	±0.08	±0.05	±0.05	±0.05
490	23	20.15	10.63	9.52	1.10	0.24⁻	1.31⁺	1.76	0.90	0.86	0.04+
±6	±2	±0.23	±0.12	±0.10	±0.04	±0.04	±0.03	±0.07	±0.04	±0.04	±0.05

[1] Excess of anions is indicated by a superior "minus" sign following the figures; excess of cations by a superior "plus" sign.

[2] Girls.

[3] SE_m represents the standard error of the mean and is the standard deviation divided by the square root of the number of observations.

out the observation, though at a greater rate during the latter period.

It is evident that the amounts and proportions in which the different elements are deposited in the body either determine or are determined by the type of construction occurring in the organism at a given time; thus, quantity intake or proportion may control the retention of minerals but many additional influences are also operating. Muscular, glandular, and neural tissues may be forming at a much greater rate at one time and skeletal tissue, containing the major amounts of the body's basic elements, at another. For this reason inquiry must be made into the reaction of the individuals to the addition of banana, to the substitution of banana for cereal, and to the substitution of banana for cereal under two different conditions owing to varied proportions of raw apple and raw banana in the total mixed diets.

The three children (group I) who received an additional 100 gm. of banana in their mixed diet (200 gm. total) during the experimental period showed average increases in daily retention of nitrogen (3 to 14 mg.), cations (0.6 to 0.8 meq.), anions (0.44 to 0.94 meq.), and total mineral ions (1.04 to 1.74 meq.) per kilogram of body weight. It is significant that the supplementary fruit enhanced the nutritive advantage of the diet in spite of the fact that the nitrogen intakes were reduced for two of the three children. With their nitrogen intakes lowered 8 and 12 mg. per kilogram of body weight, their storage of nitrogen increased from 4 to 20 and 18 mg. per kilogram, respectively. Since the accumulation of nitrogen is a good index of the growth of muscle and soft tissues it is evident that all of the children were constructing new tissue but the rate was greater during the experimental period in spite of a reduced nitrogen intake in two of the subjects (H.H. and F.C.). Although there was no weight increase by the third child (D.P.) the small amount of nitrogen stored was no doubt used in the building of the organic matrix in skeletal growth.

Daily energy intakes were only increased 1 to 3 calories per kilogram of body weight during the experimental period, a

quantity which would not greatly influence nitrogen metabolism. It is of significance to note that all three subjects (group I) gained in height and weight with the exception of D.P., who lost weight during the experimental period although he did store some nitrogen. However, his increase in height exceeded the rest of the group. The fact that there was augmented rate of growth during the experimental period is further verified by the storage of total mineral ions by all three subjects, the quantity being doubled for D.P., whose gain in height was greatest. Furthermore, on a unit weight basis these children in group I all retained an excess of cations during the pre-experimental period (13–19 meq.) but when additional banana was given H.H. and F.C. increased particularly their rates of soft tissue synthesis, indicated by greater retentions of nitrogen and retention of excess anions (0.20 and 0.30 meq. daily). D.P. continued to store a large excess of cations (0.16 meq.) daily per kilogram of body weight, 80% of which were used for skeletal growth, additional evidence that structure composition predominated over soft tissue growth in this child during the study.

The controlling factor in the different responses of H.H. and F.C., which indicated a stimulation of soft tissue growth, and that of D.P., which indicated accelerated skeletal growth, is evident in the ion balances for the urine and feces. The urine of H.H. and F.C. contained excesses of 0.62 and 0.67 meq. of anions, respectively, during the pre-experimental period. These excesses were changed to 0.04 and 0.02 meq. of cations during the experimental period, whereas the excess anions in the urine of D.P. were only reduced from 0.48 to 0.39 meq. In the feces H.H. and F.C. only increased their excretion of excess cations from 1.21 and 1.25 to 1.24 and 1.31 meq., respectively, but D.P.'s excretion increased from 0.84 to 1.06 meq. per kilogram per day.

Substitution of 100 gm. of raw banana for 30 gm. of cereal (10 gm. white bread; 20 gm. shredded wheat or corn flakes) in the mixed diets of the five children in groups II and III produced decreases in the daily nitrogen intakes per kilogram of

body weight which were accompanied by decreased retentions of nitrogen for the three children in group II, and increased nitrogen retentions for the children in group III. On a unit weight basis, with the total mineral ash values of the intakes little changed but with their alkaline-ash values approximately doubled, all five children continued to store mineral ions and four increased their rate of retention. One child (R.S.) who showed by far the largest reduction in rate of nitrogen retention of the three children in group II showed a slower rate of mineral accumulation during the experimental period.

Together, the five children responded similarly to the dietary substitution in their retention of positive and negative minerals. With the exception of the one subject whose rate of storage of total mineral ions was decelerated, the children increased their retentions of both cations and anions. In all five cases the retention of cations in relation to anions was increased, the rate of retention of an excess of cations was increased for all three children in group II and the rate of retention of excess anions was reduced for both children in group III. The increased storage of excess cations by the children in group II, together with reduced nitrogen retentions, indicates a greater effect of the altered diet toward skeleton development. While the children in group II were storing nitrogen and an excess of cations during the pre-experimental period, the subjects in group III were storing an excess of anions, indicating that they were forming a preponderance of soft tissue. The dietary change produced a reduction in these retentions but the excess retentions continued to be of anions and the nitrogen retentions increased. Apparently these differences from the response of group II were due, at least in part, to the variations in the apple and banana contents of the two groups, making the nutritive advantage of the substitution greater for the children who were receiving no banana and 200 gm. of apple during the pre-experimental period. This interpretation is strengthened by the ion balances in the excretion. All of the children in both groups reduced their excretion of an excess of anions in the urine and those

in group III reduced their excretion of excess cations in the feces; however, two of the children in group II (including R.S., whose retention of total minerals was reduced) showed considerable increases in fecal excretion of excess cations and the other child little change. These metabolic responses and changes in growth are paralleled by the changes in body weight and recumbent length.

One child (group IV) received an addition of 100 gm. of banana, 10 gm. of butter, 30 gm. of potato and 50 gm. white bread during the experimental period. The increases in intakes of cations and anions on a unit basis were approximately equal but the retention of anions increased more than twice that of cations during the experimental period, producing a shift in total retention from an excess of cations to an excess of anions. During the pre-experimental period this child showed an average loss of 6 mg. of nitrogen per kilogram of body weight per day; during the experimental period there was an average daily retention of 38 mg. per kilogram. While the data clearly show the effects of the increased intake they also indicate that the characteristics of the changed intake contributed the necessary elements for both types of tissue construction. The chemical composition of growth and the kind of nutrition were very different in the two periods and demonstrate what extensive adjustments a healthy child can make in response to a change in diet.

From the responses of the children it is evident that there are factors other than the quantity intake of positive and negative minerals and the proportion between the ions consumed in the food which influence the type of tissue formation and storage of positive or negative minerals. Moreover, they demonstrate that selective retentions take place in spite of caloric value, mere quantity intakes of nitrogen, positive and negative minerals, or the acid- or alkaline-ash values of the diet and therefore may determine the type of growth present at any one time (i.e., soft or hard tissue formation). Explanations for the type of response that a child gives to a specific dietary change must be sought in the effects of altering the interrela-

tionship of individual mineral constituents or other components of the diet at the same time intakes of nitrogen, cations, anions, and excess ions are varied. The proportion of the different positive (calcium, magnesium, potassium, sodium) and negative (phosphorus, sulfur, and chlorine) mineral elements in the diet may have more importance in metabolism during growth than total quantities of cations, anions and excess ions; or perhaps the growth impulse inherent in the individual may govern the type of tissue formation at any one time rather than the acid-ash and alkaline-ash values of the diet. The inclusion of more banana in the diet did enhance nitrogen, cation and anion retentions, results which cannot be explained on the basis of the quantity intake of these constituents, or by the proportions of excess alkaline-ash consumed, results which corroborate the observations of Roberts and associates ('39).

SUMMARY

1. Nine children, 5 to 8 years of age (four girls and five boys), who, through intensive physiologic and medical observations, were known to be in favorable nutritive condition, were the subjects of metabolic study during pre-experimental and experimental periods ranging from 20 to 55 consecutive days for each child, a total of 640 days.

2. Chemical analyses demonstrate that a conservative substitution of such foods as apple, banana, and cereal in an otherwise constant daily mixed diet on the basis of either the total mineral ash value, the total positive minerals, the total negative minerals, or even doubling the alkaline-ash values, cannot be effected without changing the proportions of other equally important dietary components such as calories, nitrogen, fat, and the proportions of the inorganic elements within the positive or negative mineral groups, any one of which might change the trend of metabolism and subsequent retentions.

3. One hundred grams of banana (one medium sized) was more effective than the same amount of apple or 30 gm. cereal

in stimulating the rate of growth as judged by increases in the individuals' height and weight, and retentions on a unit weight basis of grams nitrogen and milliequivalents of total mineral ions. This growth performance was accomplished in spite of the maintenance on a unit weight basis of approximately a constant daily intake of total positive and negative minerals during both the high and low banana periods, and an alkaline-ash value for the intakes almost doubled during the experimental period. In some subjects the type of growth stimulated was toward an equal development of bony and soft tissues; in others the trend was stronger toward soft tissue (increased storage of nitrogen with either an increased retention of anions or a reduction in retention of cations); and in others greater skeletal formation was initiated (increased storage of cations).

4. Since the dietary changes did not significantly alter the total mineral intakes, these results verify Shohl's ('39) thesis that ". . . . individual elements, or rather, certain groups of elements, perform separate functions in the body economy, and are therefore more important than the total."

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FURTHER STUDIES ON THE EFFECTIVENESS OF ARSENIC IN PREVENTING SELENIUM POISONING [1]

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ONE FIGURE

(Received for publication January 11, 1940)

Recent work at this laboratory (Moxon, '38; Moxon and DuBois, '39) has shown that arsenic will counteract selenium poisoning. It is the purpose of this paper to report further research along this line. This work was carried out on rats as an extension of that already reported.

It has been concerned mainly with the effectiveness of different forms of arsenic in preventing the toxic action of selenium in the form of seleniferous wheat and the effectiveness of arsenic as sodium arsenite in preventing the toxicity of different forms of selenium.

Different forms of arsenic

The effectiveness of different arsenic compounds was compared in controlled experiments with albino rats. The diet used and the management of the animals has been described in a previous publication (Moxon and DuBois, '39). Arsenic compounds were fed according to table 1.

Sodium arsenite (Na_2HAsO_3) and sodium arsenate (Na_2HAsO_4), when used as sources of arsenic, were equally effective in preventing the toxic action of the selenium in the diet (groups 3 and 4 of table 1) as indicated by growth, mortality rate and pathological condition of the livers.

[1] Approved for publication by the Director of the South Dakota Agricultural Experiment Station as contribution no. 126 of the Journal Series.

Arsenic at this level (5 p.p.m. in the drinking water) did not give full protection against the 14 p.p.m. of selenium in the diet. It has been shown, however, that arsenic at this level will protect rats against 11 p.p.m. of selenium in the diet (Moxon and DuBois, '39), and 10 p.p.m. of arsenic as sodium arsenite protected the rats in group 9 against 18 p.p.m. of selenium in the diet as is shown in figure 1.

In group 5 (table 1) the 10 p.p.m. of arsenic as sodium arsenite, mixed in the diet, prevented the development of symptoms of selenium poisoning slightly better than 5 p.p.m.

TABLE 1

Effectiveness of different forms of arsenic against selenium found in wheat

GROUP OF RATS	SELENIUM USED (IN DIET)	ARSENIC USED
1	14 p.p.m. (seleniferous wheat)	None
2	14 p.p.m. (seleniferous wheat)	None
3	14 p.p.m. (seleniferous wheat)	5 p.p.m. as Na_2HAsO_3 in drinking water.
4	14 p.p.m. (seleniferous wheat)	5 p.p.m. as Na_2HAsO_4 in drinking water.
5	14 p.p.m. (seleniferous wheat)	10 p.p.m. as Na_2HAsO_3 in diet.
6	14 p.p.m. (seleniferous wheat)	10 p.p.m. as AsS_2 in diet.
7	14 p.p.m. (seleniferous wheat)	10 p.p.m. as AsS_3 in diet.
8	18 p.p.m. (seleniferous wheat)	None
9	18 p.p.m. (seleniferous wheat)	10 p.p.m. as Na_2HAsO_3 in drinking water.

of arsenic, as the same compound, when fed in the drinking water (group 3). The level of arsenic intake with a diet containing 10 p.p.m. of arsenic is slightly higher than the level of intake when rats are given 5 p.p.m. of arsenic in the drinking water which will account for the greater protection offered by the arsenic in group 5 than in group 3.

The arsenic sulfides (AsS_2 and AsS_3) used in groups 6 and 7 failed to give any indication of protection against the selenium in the diet. The mortality rate in these two groups was equal to that in group 2 where no arsenic was fed with the selenium.

As a continuation of the study, "the influence of arsenic and certain other elements on the toxicity of seleniferous grains" (Moxon and DuBois, '39), lead as lead acetate and bismuth as bismuth subnitrate were fed at levels of 5 p.p.m.

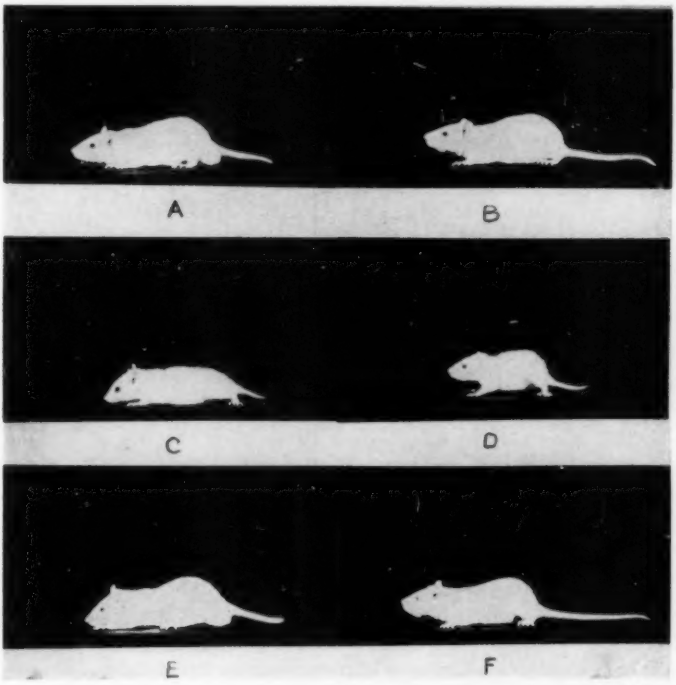

Fig. 1 A, C and E, males; B, D and F, females. A and B, control diet; C and D, seleniferous diet (18 p.p.m. Se); E and F, seleniferous diet (18 p.p.m. Se) + 10 p.p.m. arsenic in drinking water.

in the drinking water to rats on a diet containing 14 p.p.m. of selenium. In these experiments the lead did not influence the toxicity of the selenium during the duration of the feeding trials (100 days) but bismuth had a slightly beneficial effect.

Different forms of selenium

In a later experiment the efficacy of arsenic as Na_2HAsO_3 in preventing the toxic action of different forms of selenium was determined. The diet used was the same as in the above experiments. Selenium and arsenic were fed as shown in table 2.

TABLE 2

Effectiveness of arsenic as sodium arsenite against selenium in wheat and in sodium selenite

GROUP OF RATS	SELENIUM USED (IN DIET)	ARSENIC USED (IN DRINKING WATER)
1	None	None
2	11 p.p.m. Se (seleniferous wheat)	None
3	11 p.p.m. Se (seleniferous wheat)	5 p.p.m. As
4	11 p.p.m. Se (Na_2SeO_3)	None
5	11 p.p.m. Se (Na_2SeO_3)	5 p.p.m. As

The rats in group 2 were all dead within 35 days while those in group 4 were all dead at the end of 60 days. All rats in groups 3 and 5 were still alive at the end of 100 days when the experiment was terminated. The growth curves and condition of the livers on autopsy indicated that the arsenic as arsenite (Na_2HAsO_3) gave full protection against the toxic action of selenium as seleniferous wheat and as sodium selenite.

In studies involving the comparative toxicities of selenium as selenium-cystine, sodium selenite and seleniferous wheat[2] we have found that 10 p.p.m. of arsenic as arsenite (Na_2HAsO_3) will give adequate protection against 18 p.p.m. of selenium as selenium-cystine.

The response to arsenic after selenium had been fed for various periods of time

Soon after it was found that arsenic would protect against selenium poisoning (Moxon, '38) we became interested in determining what effect arsenic would have on animals in

[2] Unpublished data, this laboratory. 1938.

various stages of selenium poisoning. Six groups of rats were used as indicated in table 3.

The results indicate that arsenic is quite effective if selenium has been fed less than 30 days or not more than 20 days before the arsenic treatment is initiated. When selenium had been fed for 30 days before arsenic feeding began there was little growth response (compare groups 2 and 6 in the last column of table 3.

Liver glycogen levels have been determined (Potter, DuBois and Moxon, '39) on rats which had been fed selenium and selenium plus arsenic. Rats which had been fed a selenium diet had a lower glycogen level than the controls or the selenium plus arsenic groups.

TABLE 3

The response to arsenic after selenium has been fed for varying periods of time

GROUP OF RATS	DIET	ARSENIC (IN DRINKING WATER)	AVERAGE WEIGHTS AT 30 DAYS	AVERAGE WEIGHTS AT 90 DAYS
1	Control (no selenium)	None	115	190
2	18 p.p.m. selenium (wheat)	None	91	128
3	18 p.p.m. selenium (wheat)	10 p.p.m. As from beginning	109	187
4	18 p.p.m. selenium (wheat)	10 p.p.m. As after first 10 days	105	183
5	18 p.p.m. selenium (wheat)	10 p.p.m. As after first 20 days	102	174
6	18 p.p.m. selenium (wheat)	10 p.p.m. As after first 30 days	88	131

The protein content of diets used in selenium studies is of extreme importance. It has been shown (Moxon, '37; Smith, '39) that high protein diets offer more protection against selenium poisoning than low protein rations. The same basal diet has been used for all of our selenium-arsenic studies on rats. It contains approximately 22% protein ($N \times 6.25$).

Although some interesting clues are being followed, no explanation for the antagonistic action of selenium and arsenic can be offered at present. A similar antagonistic effect between elements has been reported for thallium and iodine.[3]

[3] Research item, Iodides as antidotes in thallium poisoning, Nature, vol. 142, p. 440. 1938.

SUMMARY

Sodium arsenite and sodium arsenate were equally effective as sources of arsenic for preventing the toxic action of the selenium present in seleniferous wheat in the diet of albino rats; the arsenic sulfides (AsS_2 and AsS_3) were ineffective.

Arsenic as sodium arsenite was equally effective against selenium when the latter was given as seleniferous wheat, sodium selenite and selenium-cystine.

Arsenic was effective in treating rats which had been fed selenium for 20 days but was of little value after selenium had been fed for 30 days.

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NICOTINIC ACID POTENCY OF FOOD MATERIALS AND CERTAIN CHEMICAL COMPOUNDS [1]

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ONE FIGURE

(Received for publication January 6, 1940)

The extensive use of nicotinic acid in the treatment of human pellagra (Spies, Bean and Ashe, '39) renders a knowledge of its quantitative distribution in various food materials of considerable interest. A number of assay methods, both chemical and biological, have been worked out, but quantitative data have been made available only through use of the chemical methods.

The chemical methods available at present for the determination of nicotinic acid in biological materials give varied results. Colorimetric methods have been reported by Swaminathan ('38), Karrer and Keller ('39), Kringstad and Naess ('39), Bandier ('39) and Harris and Raymond ('39). The greatest difficulty encountered is the complete extraction of the active compounds from the materials investigated. Since little information is available on the actual form of nicotinic acid in naturally occurring materials, the best method of extraction of this compound must await further investigation. The colorimetric determinations are further beset by the disadvantage that they require a colorless extract for the most convenient development of color. Any effort to adsorb

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extraneous pigment from an extract may bring about the adsorption of nicotinic acid as well, thus giving low values.

The biological method depends on the treatment or prevention of the specific disease black tongue in dogs. Using the black tongue preventative technique Goldberger et al. ('28) and Sebrell et al. ('34, '35) were able to determine qualitatively the distribution of the anti-pellagra factor in a number of foodstuffs. The use of dogs for bio-assay purposes has the advantage of furnishing information as to the actual nicotinic acid potency of a particular food, regardless of the form in which nicotinic acid occurs. During the past 2 years we have used this bio-assay for determining the nicotinic acid potency of (1) meats and meat products, (2) other food materials, and (3) certain specific chemical compounds related to nicotinic acid.

Mongrel dogs were used in all of the assays.[2] They were placed on a modification of the Goldberger ration (Goldberger, Wheeler, Lillie and Rogers, '28) having the following composition:

	%
Ground yellow corn	71
Purified casein [3]	18
Salts [4]	4
Cottonseed oil	5
Cod liver oil	2

Since similar rations have been shown to be deficient in riboflavin and thiamin (Helmer and Fouts, '38; Margolis, Margolis and Smith, '39) in addition to nicotinic acid, these two pure vitamins were given weekly in aqueous alcoholic solutions at a level of 50 mcg. per kilogram of body weight per day.[5] When it appeared likely that corn contained less of vitamin B_6 than was previously supposed, vitamin B_6 [6] was also given twice weekly in dosages of 500 mcg. per dog.

[1] We are indebted to the Works Progress Administration project no. 8649 for assistance in the care of animals.

[2] Washed eight times, reprecipitated twice.

[4] Phillips and Hart (J. Biol. Chem., vol. 109, p. 657, 1935) salt mixture with $MnSO_4 \cdot 4H_2O$ increased from 0.7 to 10 gm. per kilogram of salt mixture.

[5, 6] We are indebted to Merck and Company for generous supplies of thiamin, nicotinic acid and vitamin B_6.

The dogs developed typical black tongue in from 1 to 3 months. When the first distinct drop in weight was observed, a dog was given a standardizing dose of 20 mg. of nicotinic acid by capsule. The animals responded to the vitamin within 12 to 24 hours by beginning to eat the ration. Usually in 5 to 14 days the restoration of weight had reached its peak, and in 14 to 21 days the dogs had lost weight and reached the level at which they were standardized. The response in weight to the standard dosage of nicotinic acid was noted and the gain in weight per milligram of nicotinic acid was calculated.

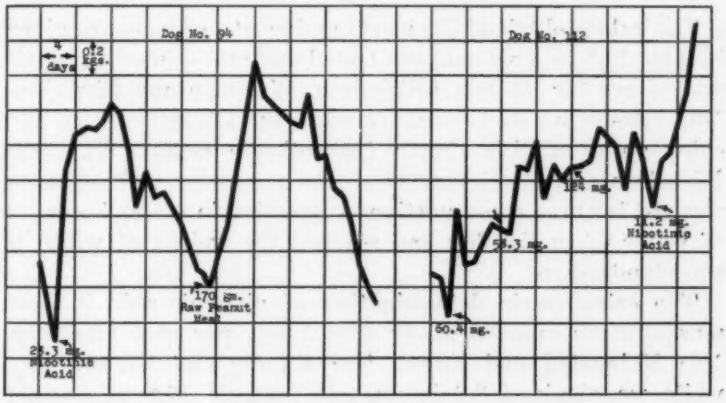

Fig. 1 Curve 1. Standardization with nicotinic acid followed by an assay of peanut meal. Curve 2. Effect of 3 doses of pyrazine monocarboxylic acid followed by one of nicotinic acid on the growth of a young pup.

A foodstuff to be assayed was then fed to the dog at a level which would contain approximately the same amount of nicotinic acid as the original standardizing dose. The weight response elicited by the supplement was noted and by a simple proportion the amount of nicotinic acid per gram of material was calculated. A typical response to a standard amount of nicotinic acid followed by an assay of peanut meal is shown in figure 1.

Repeated attacks of black tongue in a dog may decrease the animal's efficiency for assay purposes due possibly to gastro-

intestinal lesions. Some indication of the fact that certain dogs have a decreasing ability to respond to a supplement was obtained in a few animals and the assays in these cases were discarded. In order to minimize any error each animal was restandardized after two successive assays by again giving 20 mg. of nicotinic acid by capsule to the dog. To further increase the accuracy of the assays a foodstuff was usually fed to more than one dog or was reassayed on the same dog at a later period.

RESULTS

The values obtained for meats and meat products are given in table 1. The meat samples tested were those used in previous assays for thiamin (Mickelsen, Waisman and Elvehjem, '39), riboflavin (Mickelsen, Waisman and Elvehjem, '39), and chick antidermatitis factor (pantothenic acid) (Waisman, Mickelsen and Elvehjem, '39). Each sample of dried meat was fed in three equal portions suspended in a small amount of water when the dog had reached the weight at which it was standardized.

The colorimetric determination of nicotinic acid in beef muscle gives approximately 4 to 5 mg. per cent (Bandier, '39; Kringstad and Naess, '39; Karrer and Keller, '39), while we obtained 3.8 in one sample and 10.2 in another sample of the same tissue. Bandier ('39) and Kringstad and Naess ('39) found 4.7 and 3.3 mg. per cent respectively in pork muscle, while our values ranged from 5.3 to 13.0 mg. per cent. The beef spleen analyzed by Bandier ('39) was slightly lower than the range of nicotinic acid found in our samples of the same tissue. The values for pork heart and beef heart found by Bandier check within the range found in our samples. Whereas Bandier ('39) found 6.5 mg. per cent for beef kidney and 6.8 for pork kidney, Karrer and Keller ('39) found 19.4 for beef kidney which agrees well with 16.9 found in our sample of beef kidney, and with 15.5 mg. found in our sample of pork kidney. Kringstad and Naess ('39) found 18.0 mg., Bandier ('39) found 12.2 mg., and Karrer and

TABLE 1

Nicotinic acid content of meats and meat products

SAMPLE	SAMPLE NUMBER	MILLIGRAMS OF NICOTINIC ACID PER 100 GM. OF THE FOOD			
		Dry	Fresh	Fresh, as reported in the literature	
Pork liver	33	90.0	26.4	11.8	(Bandier, '39)
Pork liver	122	110.0	27.5		
Beef liver	110	110.0	27.5	9.3	(Karrer and Keller, '39)
Beef liver	120	85.0	25.0	12.2	(Bandier, '39)
Beef liver, fried	121	87.0	29.4		
Veal liver	70	72.0	22.5	18.0	(Kringstad and Naess, '39)
Lamb liver	61	131.0	46.0		
Lamb liver	96	135.0	39.2		
Pork kidney	62	72.0	15.5	6.83	(Bandier, '39)
Beef kidney	84	81.0	16.9	6.5	(Bandier, '39)
				19.4	(Karrer and Keller, '39)
Beef kidney	126	89.0	17.8		
Pork heart	71	32.0	8.0	5.34	(Bandier, '39)
Beef heart	73	23.0	4.9	5.93	(Bandier, '39)
Beef spleen	76	28.0	7.0	4.42	(Bandier, '39)
Beef spleen	108	33.0	8.3		
Beef spleen	123	52.0	12.3		
Pork ham	95	40.0	9.7	4.73	(Bandier, '39)
Pork ham	32	38.0	10.4	3.3	(Kringstad and Naess, '39)
Pork ham	114	37.0	10.0		
Pork ham	118	32.0	8.8		
Boiled ham	115	15.0	5.2		
Smoked ham	116	28.0	8.2		
Tenderized ham	119	25.0	8.3		
Pork loin	74	46.0	13.0		
Pork loin	92	25.0	7.5		
Pork loin	125	19.0	5.3		
Beef tongue	82	46.0	12.8		
Veal hindquarter	75	70.0	16.1		
Veal hindquarter	44	72.0	18.0		
Veal hindquarter	103	24.0	6.5		
Beef brain	77	30.0	7.5		
Beef muscle	40	15.0	3.8	4.1	(Bandier, '39)
Beef muscle	105	37.0	10.2	3.8	(Karrer and Keller, '39)
				4.9	(Kringstad and Naess, '39)
Roast beef	107	37.0	10.2		
Beef pancreas	79	11.0	2.7		
Beef pancreas	64	16.0	3.5		
Beef lung	78	33.0	8.3		

Keller ('39) found 9.3 mg. in their samples of beef liver while our values were somewhat higher. Our assays show that pork liver contains about the same as beef liver. Bandier ('39) found less than half of our values in these tissues.

It will be seen by inspection of the data that the organ tissues are richer in anti-black tongue activity than muscle tissue. This has been a consistent finding in the previous assays reported from this laboratory on the distribution of the various factors of the vitamin B complex in meats and meat products. Recently, work by Axelrod, Madden and Elvehjem ('39) showed that both liver and kidney of the dog and rat contained more coenzyme I (cozymase, nicotinic acid containing coenzyme) than the muscle and brain tissues of these animals. Their work demonstrated that pig kidney and brain maintained their normal coenzyme I content but decreases were always noted in the liver and muscle in nicotinic acid deficiency. Although no positive correlations could be made from their work, there was an indication that the coenzyme I content was a function of the degree of the deficiency. The tissues used for assay may then be expected to vary in their nicotinic acid content depending upon the state of nutrition of the animal.

In table 2 the values for food materials other than meats are listed. The values for yeast obtained with this method compare favorably with those reported by Swaminathan ('38), Bandier and Hald ('39), and Kringstad and Naess ('39) who used chemical methods, but the high variation in nicotinic acid content of different yeast samples makes such a comparison difficult. The results obtained with liver extract are consistently higher than those obtained by chemical methods. Shaw and MacDonald ('38) reported a maximum of 49 mg. per cent of nicotinic acid for liver extract powder using the cyanogen bromide method, while we have obtained the value of 270 mg. per cent. Liquid liver extracts vary greatly in their method of preparation and in total solids content. Our high value of 450 mg. per cent was obtained from an alcohol soluble

liver extract representing a high concentration from fresh liver.

The value obtained for skim milk powder, however, was only approximately one-half that reported by Swaminathan ('38), while our value for unroasted peanut meal reduced to terms of his ether extracted meal compares favorably with his assay. The very low activity of eggs, wheat germ, and extracted wheat germ is of interest since these had been reported

TABLE 2

FOODSTUFF OR SOURCE MATERIAL	NICOTINIC ACID mg.%
1. Skim milk powder	4.3–6.2
2. Bakers yeast	50
3. Brewers yeast Sample A	93
B	90
C	35
D	34
E	39
F	56
4. Liver extract, alcohol soluble (Wilson Laboratories no. 36997)	450
5. Liver fraction "B" (Wilson Laboratories no. 38818)	210
6. Liver extract powder (Wilson Laboratories no. 38785)	270
7. Alcohol-ether precipitate fraction of liver extract powder	less than 35
8. Rice bran extract, a commercial	165
9. Whey concentrate, a commercial	less than 15
10. Peanut meal (raw)	13
11. Grass, a commercial preparation	7.8
12. Egg yolk (hard boiled)	less than 4
13. Egg white (hard boiled)	less than 2.5
14. Wheat germ	less than 4
15. Extracted wheat germ, a commercial	less than 6

to be fairly good sources of the factor by Goldberger et al. ('28) and their inclusion in pellagra curative diets was recommended.

The effective use of quinolinic acid in the treatment of human pellagra as reported by Vilter and Spies ('39) suggested another trial of this compound in the treatment of acute black tongue. Since a previous assay had failed orally (Woolley, Strong, Madden and Elvehjem, '38) a dose of 213

mg. was injected.[7] This produced no effect. It is therefore apparent that quinolinic acid is decarboxylated neither in the dog intestine nor by the tissues, and that it cannot be utilized as such by the tissues. The point of decarboxylation of quinolinic acid in the human body is still unknown since it was administered only by the oral route.

The efficacy of pyrazine mono- and 2, 3 dicarboxylic acids as reported by Bills, McDonald and Spies ('39) when administered orally to human pellagrins suggested a trial of these compounds. Pyrazine monocarboxylic acid was ineffective in restoring weight to or even maintaining dogs thus depleted when given orally at a level of 106 mg.; pyrazine 2, 3 dicarboxylic acid was ineffective at a level of 104 mg. and again at 195 mg.[8] All these levels were at least five times the magnitude of the standardizing dose of nicotinic acid. However, slight responses obtained with growing puppies on this ration (fig. 1) with both compounds might indicate a mobilizing effect of these compounds for nicotinic acid.

The growth stimulating effect of thiazole-5-carboxylic acid for dysentery bacilli when substituted for nicotinic acid in the basal synthetic media as reported by Schmelkes ('39) suggested an assay of this compound. Both the acid and its amide were inactive at levels of 310 and 440 mg. respectively, although another assay of the amide using 1.05 gm. indicated slight activity.[9]

DISCUSSION

It has been shown that the more recent results obtained by improved chemical methods are not greatly different from the bio-assays. In general the bio-assay gives higher figures which may be due to the fact that the animal organism will efficiently utilize all forms of nicotinic acid derivatives. While the chemical method may be limited in its reaction, it is also

[7] Furnished by Prof. S. M. McElvain of the Chemistry Department, University of Wisconsin.

[8] Furnished by Dr. Tom D. Spies.

[9] Furnished by Dr. F. C. Schmelkes of Wallace and Tiernan Company, New York.

likely that the response obtained by feeding foods such as liver, which contains practically all of the B complex vitamins, is slightly greater than the administration of nicotinic acid alone due to a more efficient utilization of the vitamin. If this is true the bio-assay may tend to give higher values than the chemical method. In any case the results presented in this paper give a relative indication of the anti-pellagra activity of the products tested.

SUMMARY

1. The biological assay for the nicotinic acid content of various food materials has been accomplished by the use of black tongue dogs maintained on the modified Goldberger diet.

2. The nicotinic acid content of meats and meat products ranges from 10 to 110 mg. per 100 gm. of dry tissue.

3. Liver extracts contained from 200 to 450 mg. of nicotinic acid per 100 gm. Yeast ranged from 30 to 100 mg. per 100 gm. dry weight.

4. Quinolinic acid was found to be inactive as a substitute for nicotinic acid when injected. Pyrazine mono- and 2, 3 dicarboxylic acid and thiazole 5 carboxylic acid were either inactive or of very low activity.

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PSEUDO-HYPOPHYSECTOMY

A CONDITION RESEMBLING HYPOPHYSECTOMY PRODUCED BY MALNUTRITION

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TWO FIGURES

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Many dietary procedures which are intended to elucidate endocrine and other physiological functions of the rat result in decreased food intake and loss in body weight. We have reinvestigated the effects of starvation and of underfeeding upon the tissues of the rat. In addition to the anestrus and sterility which developed during malnutrition, the anatomic changes observed bore a close resemblance to those following hypophysectomy. Since this operation results in a decrease in food intake and a loss in body weight, it is possible that the concomitant inanition which follows ablation of the pituitary gland may be responsible in part for some of these tissue changes. On the other hand, Jackson ('25), from his studies on inanition, suggested that ". . . . changes produced in the hypophysis may be responsible for some of the general phenomena of inanition and malnutrition." Mason and Wolfe ('30) stated that, "The hypophysis of male rats subjected to chronic inanition showed a significant decrease in activity . . . ." (See also Marrian and Parkes, '29; Moore and Samuels, '31; Werner, '39.)

The problem presented is to determine to what extent the effects of inanition on the rat are due to the primary tissue malnutrition and to what extent they are secondary to the

diminished elaboration of hormones by the malnourished endocrine glands. It is the purpose of this report to elucidate the part played by the hypophysis during inanition. Changes produced in the pituitary have been found to be at least in part responsible for many of the tissue changes occurring in inanition. The effects of inanition upon certain tissues we have termed pseudo-hypophysectomy (Pomerantz and Mulinos, '39) in order to stress their resemblance to true hypophysectomy and to distinguish them from the tissue changes due directly to the malnutrition.

PROCEDURE

Approximately 300 albino adult rats were employed for this study. Each procedure was carried out on groups of five rats of the same sex which were kept in a single cage. The food and water intake of the group was determined daily together with the body weight of each rat. Daily estrous smears were taken and also palpation for size and position of testes was made.

The rats were subjected to two types of inanition:

1. Complete inanition, induced by withholding all food and water. Two-hundred-gram rats lived approximately 10 days (7–13) under these conditions. Tissue changes were most marked just before death; they were greatest in those rats which lost the greatest percentage of body weight.

2. Chronic inanition, induced by allowing one-half of the amount of the complete food mixture [1] that the rats had been eating during a preliminary period of observation. Each rat was allowed from 6 to 8 gm. of food per day, approximately the same as that reported to be ingested voluntarily by hypophysectomized adult rats (Lee, '38).

Control groups of rats fed the full diet were always run in parallel with the inanition experiments. In this way, seasonal,

[1] Egg—Breeder Mash, Quaker Oats Co. Chemical analysis: protein 20%; fat 4.5%; fiber 8%, carbohydrates (nitrogen-free extract) 45%. Ingredients: oatmeal, hominy feed, wheat bran and middlings, barley feed, alfalfa meal, cereal grass, fish meal, meat scraps, cod liver meal, sardine oil, dried whey and buttermilk, cane molasses and 0.75% salt.

room temperature, weight, sex, and age influences were reduced to a minimum.

At the predetermined time in each experiment, the rats were anesthetized lightly with ether, the chest wall opened, the animals bled to death from the heart, and autopsy was performed immediately. In most of the autopsies the hypophysis, thyroids, thymus, spleen, liver, kidneys, adrenals, and sex organs were carefully dissected, and weighed individually on an analytical balance.

RESULTS

Since the effects of inanition in the rat were found to be essentially the same as those described by numerous investigators and thoroughly reviewed by Jackson ('25), this report concerns itself with a comparison between inanition and hypophysectomy and not with a complete description of these conditions. The evidence presented reveals that the tissues of adult underfed rats anatomically and physiologically resemble in many respects those of adult hypophysectomized rats as reported in the literature.

The adrenal glands. After about a week of complete inanition, the adrenal glands may be larger and heavier than in fed controls (fig. 1). They may be congested with blood and red in color. These changes resemble those reported by Perla ('35) to occur within the first 2 weeks after ablation of the hypophysis. Chronic inanition, however, resulted in atrophy of the adrenal cortex (fig. 1). The atrophy was never marked, and involved chiefly the cytoplasm, increasing the nucleus-plasma ratio. The findings are similar to those of Jackson ('25). These cellular changes resemble those resulting from hypophysectomy in which, "The cells of all three zones exhibit a marked diminution in their cytoplasm. The nuclei are consequently massed together. It appears that the reduction of the cortex is not affected through disappearance of cells, but rather by this diminution in their cytoplasmic content" (Smith, '30 a).

During inanition, the cytoplasm of the cells of the cortex and especially of the zona reticularis is diminished, thereby

making the nuclei appear relatively numerous, while the dark-staining chromatin is arranged in spoke-like fashion (fig. 2). Cells of this type found in the ovary have been designated "wheel cells," because of the nuclear appearance (Selye, Collip and Thomson, '33). They have not been reported as

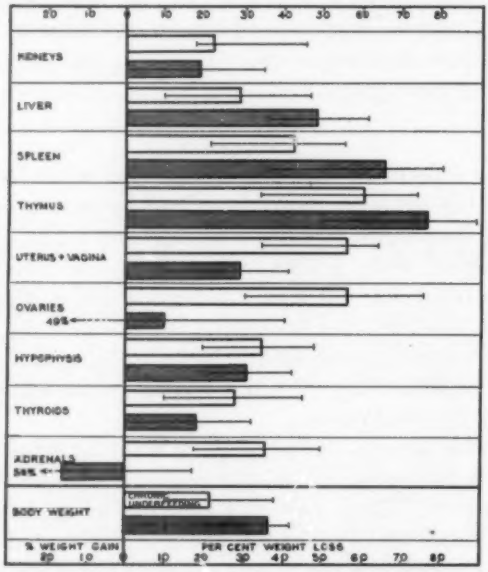

Fig. 1 Relationship between the loss in body weight and the loss in organ weight during complete (stippled bars) and chronic inanition. The chart is based upon data from a representative group of twenty normal control, thirty completely starved (5 to 11 days), and twenty-five chronically underfed (26 to 182 days) female rats. The average initial body weight was 200 gm.

The bars indicate the average percentage weight change from the normal controls, while the thin lines within the bars represent the range of variation.

occurring in the adrenal cortex of the hypophysectomized or underfed rat.

Chronic inanition in the rat is followed by deposition of a greenish-yellow pigment in the zona reticularis of the adrenal cortex and is especially marked in rats which are underfed for long periods (3 to 4 months). The pigment masses vary in size from fine particles to particles larger than the neighboring

nuclei. These pigmentary deposits are similar to those described by Cutuly and Cutuly ('37) as occurring in the adrenals of hypophysectomized rats. They believed that the pigment "represented a type of degeneration resulting after ablation of the anterior lobe of the pituitary."

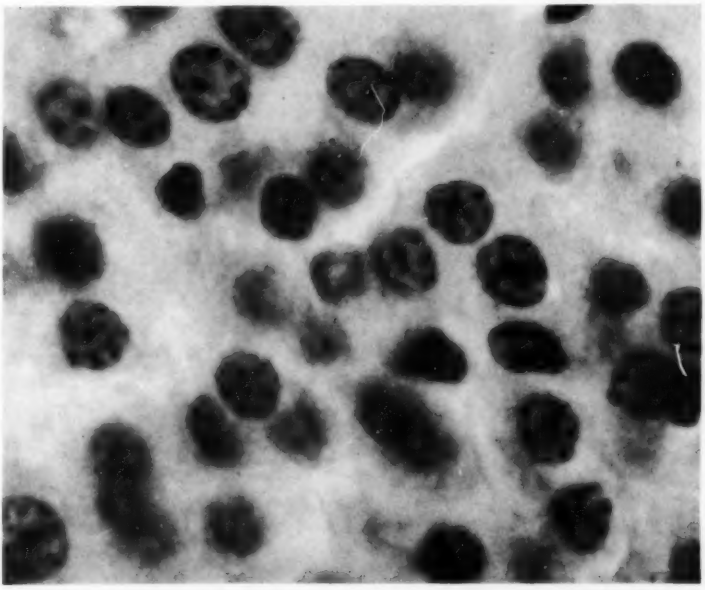

Fig. 2 Wheel cells in the cortex of the adrenal gland of a rat underfed for 42 days. Note the spoke-like arrangement of the nuclear chromatin. The animal lost 17% of its body weight and the adrenals weighed 64% (30 mg.) of the average normal adrenal weight (47.4 mg.). Similar cells appear in ovaries and interstitial tissue of the testes of chronically underfed rats.

The adrenal medulla is virtually unaffected either after hypophysectomy or during chronic inanition. It may appear relatively enlarged because of the cortical atrophy. The medullary cells retain their chromaffin reaction in both conditions.

The thyroid gland. In complete inanition the loss in the weight of the thyroid gland is relatively less than the loss

in body weight, whereas in chronic inanition it is greater (fig. 1). The atrophy from inanition is never so great as after hypophysectomy. The histologic changes are qualitatively similar, however. The cuboidal cells of the follicles are atrophied and reduced in height. The nuclei stain more deeply, and although shrunken, they appear relatively more prominent. The intrafollicular colloid persists even until the death of the rat.

The spleen. In both total inanition and chronic inanition with continued weight loss there is a decrease in the weight of the spleen proportionately greater than the loss of body weight. The atrophy is most marked under complete inanition when the weight loss may be over 60% (fig. 1). There is atrophy of the lymphoid tissue. The malpighian corpuscles are reduced in size and appear relatively numerous.

After hypophysectomy there is a similar reduction in the size of the spleen (Smith, '30 a). Perla ('36) found that 2 months after hypophysectomy the ratio of spleen to body weight fell to about one-half the normal. There was a decrease in the size of the follicles of these spleens with an increase in their number per unit area. Deposits of a greenish-yellow pigment (giving the Prussian-blue reaction) which are found in spleens of hypophysectomized rats (Kutz, McKeown and Selye, '34) also occur in the spleens of our starved or underfed rats.

The liver. In our rats, complete and chronic inanition caused a relatively greater loss in liver weight than in body weight (fig. 1). Some of the liver weight loss is due to the extreme glycogen depletion which is a part of the rapid diminution in all carbohydrate stores, and is more apparent the greater the inanition.

After hypophysectomy there is a loss of liver weight which is usually relatively greater than the loss in body weight (Smith, '30 a). Franseen, Brues and Richards ('38), and Higgins and Ingle ('39) independently noted that after partial hepatectomy the rate of liver restoration was greater in control (fully fed) than in hypophysectomized rats. However, these investigators reported that when control rats were

underfed purposely and made to lose weight parallel to the hypophysectomized animals, the rate of liver restoration was essentially equal in both groups.

The thymus. Complete and chronic inanition in our rats resulted in marked involution of the thymus gland, the percentage loss in weight being far in excess of the body weight loss (fig. 1). There was a decrease of the lymphoid elements with an apparent increase in connective tissue. In extreme cases of inanition the clear differentiation between the cortex and medulla disappeared.

According to the data presented by Smith ('30 b), the loss of thymus weight after hypophysectomy in the rat was also relatively greater than the loss of body weight. Smith ('30 b) considered the possibility that inanition may have contributed to the thymus weight changes.

Hair growth. Small areas of skin on the backs of chronically underfed and normal control rats were depilated with a barium sulfide mixture. Inanition now delayed the regrowth of hair from a few days to several months beyond the normal period; the retardation was roughly proportional to the degree of inanition. Following hypophysectomy, the growth of hair over shaved areas of skin has been reported to be slower than in unoperated fully fed controls (Snow and Whitehead, '35).

The female reproductive system. Anestrus usually occurs in adult rats during inanition (Evans and Bishop, '22) and often develops when body weight loss is only 15% (Mulinos et al., '39). Hypophysectomy is also followed by anestrus. Coincident with the loss of estrual cycles resulting from complete inanition, the number and size of the graafian follicles are decreased, yet the weight and size of the ovary are not markedly altered (fig. 1). Within 3 weeks chronic inanition usually results in changes in the structure of the ovary which are qualitatively similar to those reported to occur after hypophysectomy. Primordial follicles continued to develop throughout the period of inanition, but upon reaching the stage of antrum formation, they become atretic. Within 3 weeks the ovary has shrunken in size and contains many

atretic follicles. During this time, when there is usually a typical anestrous vaginal smear, the ovaries contain but a few degenerated corpora lutea, in contrast to the persisting corpora lutea of hypophysectomy. After 2 months of chronic inanition during which there has been no typical estrual activity, it is not unusual to find a few nucleated and cornified epithelial cells in a vaginal smear predominantly leucocytic. This is especially true in the rats which have lost least in weight. The ovaries of these rats often contain several well-formed corpora lutea. The presence of corpora lutea after more than 2 months of anestrus is indicative of the development of some follicles into corpora lutea, but in number too few to yield sufficient estrogenic hormone for the appearance of a typical estrous smear. The elaboration of estrogenic hormone by such ovaries is reflected by the fact that the adnexa of these animals are heavier than those in the completely anestrous rats.

Around the degenerated follicles of the ovary of the hypophysectomized rat there are thecal cells which are poor in cytoplasm and contain a "wheel-like" nucleus not unlike that of a plasma cell. These "wheel-cells" have been described by Selye et al. ('33) as being ". . . . a typical deficiency reaction of the ovary after hypophysectomy . . . ." Cells indistinguishable from these "wheel-cells" have been found by us to occur in the ovaries of rats during chronic inanition (Pomerantz and Mulinos, '39). The number of "wheel-cells" is related to the degree and duration of the inanition. If it be allowed that inanition decreases the hormonal activity of the anterior pituitary gland, a common etiology for the origin of the "wheel-cells" of hypophysectomy and chronic inanition becomes evident.

The loss in adnexal weight is progressive and as the period of inanition lengthens, the weight loss becomes relatively greater than the body weight loss (fig. 1). The greater the atrophy of the ovary from inanition, the greater is the atrophy of the adnexa. Pituitary substance (Marrian and Parkes, '29), gonadotropic hormone (pregnancy urine extract) or estrogen (Mulinos et al., '39) injected into chronically

underfed animals result in adnexal hypertrophy and the appearance of estrus. The adnexa of hypophysectomized rats respond in a similar manner to the injection of these hormones. An insufficiency of estrogen appears to be common both to inanition and to hypophysectomy and thus the cause of the anestrus and adnexal atrophy.

The male reproductive system. Complete inanition unto death results in the destruction of an occasional seminiferous tubule, whereas prolonged chronic inanition results in severe atrophy of the testes and accessory genitalia. After chronic inanition the changes in the testes are qualitatively similar to those described as occurring in the adult rat after hypophysectomy (Smith, '30 a). The atrophy is more severe in the latter but the results are complicated by the fact that the testes are pulled up into the abdomen soon after hypophysectomy. After 50 days of chronic inanition the testes and accessories may weigh less than one-fifth the normal despite the fact that the testes remain in the scrotum. The production of spermatozoa is abolished after hypophysectomy and diminished or absent during inanition. There is generally no destruction of the Sertoli cells or the spermatogonia in either condition. There appears to be no hypertrophy of the interstitial tissue concurrent with the tubular degeneration due either to inanition (Siperstein, '21) or to hypophysectomy (Smith, '30 a). Stained sections of the testes of rats with chronic inanition contain many interstitial cells with "wheel-like" nuclei which resemble those occurring in the adrenal cortex and the ovarian stroma. These cells have not been described in the atrophic testes of the hypophysectomized or the underfed rat.

The hypophysis. The hypophysis lost in weight relatively less during acute than during chronic inanition, the loss in the latter being relatively greater than the body weight loss. Histologically, the gland showed evidence of atrophy as already described by Jackson ('25). The atrophy of the hypophysis may result in an impairment of function. Werner ('39) has shown that the hypophyses of chronically underfed

rats contain less gonadotropic principles than those of normally fed rats.

DISCUSSION

The responses of the various organs of the body to severe and continued inanition may be divided into three groups or degrees:

Group I. The most obvious effects of inanition are those due to direct malnutrition from a deficiency in calories, minerals, vitamins, etc. In this first group probably belong the kidneys, and other structures, the loss of weight in which is usually proportional to the loss in body weight.

Group II. A secondary effect of inanition may be expected to affect organs which are dependent for their full activity upon hormones elaborated elsewhere. For example, the pituitary gland may respond to inanition by the primary reaction described under group I with a fall in the quantity and perhaps the quality of its secretions. This results in a diminution of the concentration of gonadotropic principle to the end that a primarily malnourished ovary will receive less stimulation than normally, and become secondarily depressed. The ovarian response to inanition may, therefore, be a loss in weight greater in proportion than the loss in body weight, a phenomenon which actually occurs. The testes and adrenals, and perhaps the thyroid and spleen, are some of the organs which may be secondarily affected by the primary effect of inanition upon the anterior pituitary gland.

Group III. Following the reasoning under group II, a tertiary effect may result from the secondary. An example is offered by the response to inanition of the sex accessory organs of male and female rats. The factors involved in this response would be threefold: (a) direct malnutrition of the accessories themselves, (b) diminution of the secretion of accessory-stimulating sex hormone by the poorly nourished gonads, and (c) further diminution of gonadal secretion due to the malnutrition of the pituitary gland, and the consequent fall in the secretion of gonadotropic hormone.

The tissue changes resulting from underfeeding are of the greatest importance in the consideration of data from experiments purporting to elucidate certain problems. In any experimental procedure which results in a loss of body weight one must take into account the effects of the concomitant inanition. As far as the rat is concerned malnutrition complicates all toxicological experiments, and many dietary ones, especially those in which vitamin B_1 is lacking. All such experiments must be run in parallel with controls which are made to lose in weight at the same rate as the experimental animals. Finally, it is essential to distinguish between acute and chronic inanition since the organ changes are often qualitatively dissimilar in the two conditions.

SUMMARY

Data have been obtained from over 300 rats upon acute and chronic inanition, and then compared with the tissue changes reported to occur after hypophysectomy. The effects of prolonged chronic inanition resemble those from hypophysectomy, especially upon the endocrine organs. The endocrine tissue changes are qualitatively similar in the two conditions but they are seldom so marked during chronic inanition as they are following hypophysectomy. Other tissue changes are equally marked in both conditions and appear to be related to the loss in the body weight.

The response of the endocrine organs of the rat to chronic inanition has been termed pseudo-hypophysectomy not only because of the resemblance between the effects of inanition and hypophysectomy but also because we believe that many of the effects of inanition are due to malnutrition of the hypophysis, resulting in a diminished secretion of hormones.

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PROTEIN ANABOLISM IN THE ORGANS AND TISSUES OF PREGNANT RATS AT DIFFERENT LEVELS OF PROTEIN CONSUMPTION [1]

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ONE FIGURE

This investigation is a sequence to a previous study on the distribution of protein in the body of the pregnant rat (Poo, Lew and Addis, '39). It was required because we made two observations we could not comprehend. One was the fact that the concentration of protein in the serum, carcass, liver, blood clot, kidney and heart was reduced relatively to non-pregnant controls. Since the rats had been maintained on a diet that did not contain much protein we were not sure whether this decreased protein concentration was an intrinsic characteristic of the pregnant state or was indicative of a relative protein deficiency arising because in pregnancy the rate of protein anabolism was more than double that of non-pregnant controls. The other observation was that the proportion of the total maternal protein assigned to the liver during pregnancy was much greater than in the controls. In this case two possible explanations occurred to us: this might be due to a work hypertrophy of the liver in pregnancy or, much less probably, to an accumulation of protein to be used later during lactation. By making similar measurements of protein distribution in pregnant rats kept on diets that varied widely in protein content one can determine whether this increased allocation of

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protein to the liver is independent of or related to the supply of food protein. Under such conditions one can also answer the question as to the significance of the decrease in tissue protein concentration in pregnancy since at the higher levels of protein consumption there will be no possibility of an inadequate protein intake. Under these conditions further information will be obtained with respect to the extraordinary changes that occur during pregnancy in the internal distribution of protein within the body. It is as though the uterus and its contents became a parasite on the mother and deflected to itself protein normally apportioned to other parts. We had observed, for instance, that only 76% of the total protein in a pregnant rat was found in the carcass (mainly muscle, skin and skeleton) as compared with 86% in non-pregnant rats subsisting on the same 16% protein diet. It is of practical importance for the dietetics of pregnancy to determine whether such deflections from the usual assignment of protein to the various organs and tissues of the body may be diminished or abolished by increasing the supply of food protein.

A number of female rats about 90 days of age that had never borne litters were taken from a colony that had been reared on a diet containing 18% of protein. They were kept with males for 5 days and those that were pregnant were divided into four groups in such a manner that at the beginning of the experiment the average body weight of each group was 150 gm. and in each case the distribution of individual body weights was similar. On this zero day each group was given a diet that differed with respect to its protein content but was otherwise the same. The four diets contained respectively 11%, 16%, 27% and 43% of protein. This variation was accomplished by having each diet contain 60% by weight of a mixture of cornstarch and commercial casein that had a 79.2% protein content. The diet with the lowest protein concentration had 10% of casein, the next 16%, the next 30% and the highest 50% of casein, the remainder of the mixture being made up to 60% with cornstarch. In addition all the diets contained 15% of lard, 10% of sardine oil, 9% of dry yeast, 2% of dry

alfalfa and 4% of Osborne and Mendel's salt mixture. After 18 days on these diets, when pregnancy on the average had continued for 20.5 days and delivery was imminent, the four groups were anesthetized, exsanguinated, and the protein content of their organs and tissues was determined. In order to ascertain how much protein had been formed during these 18 days another group of twenty non-pregnant rats whose average body weight was 150 gm. was killed at 0 days, i.e., the day on which the various diets were first given to the pregnant groups, and the protein content of their organs and tissues was measured. The methods used were those described by Addis, Poo, Lew and Yuen ('36). The quantities found and the corresponding fresh weights are given in table 1.

The survey of the results in table 1 will be facilitated by reference to figure 1 in which the amounts of newly formed protein are expressed as percentages of the amounts found at 0 days.

The curves for total protein show how great are the changes in anabolism that can be induced by varying protein consumption. When only about a gram of food protein per day is taken, the total anabolism is even less than in non-pregnant controls, although with any adequate protein intake the quantity of new protein formed is much greater than in non-pregnant controls. In both pregnant and non-pregnant rats, however, the highest rate of anabolism occurs when about 2.5 gm. of protein are eaten and in both, anabolism is decreased when larger amounts are consumed.

What we have termed "maternal protein" in figure 1 is the total body protein minus the protein of the uterus and embryos. If pregnancy had no effect on the mother the pregnant and control curves should be superimposed; not only are they divergent but they cross one another, indicating that the nature of the effect is dependent on the amount of protein that is consumed. When the amount taken is small the mother's tissues lose protein, i.e., the relation between the mother and the uterus and its contents is one of host and parasite. When larger amounts are taken the mother's tissues construct more

TABLE 1

Fresh weight and protein content per rat of organs and tissues before and after 18 days of increasing protein consumption during pregnancy

CATEGORY OF INTEREST [1]	BEFORE PREGNANCY AT "ZERO DAYS" ON STOCK DIET	DURING PREGNANCY AFTER 18 DAYS SUBSISTENCE ON DIETS VARIED WITH RESPECT TO PROTEIN			
Protein content of diet in per cent	18	11	16	27	43
Protein eaten per rat per day in gm.	..	1.04	1.95	2.89	4.44
Body weight—total in gm.	150.000	191.333	232.301	228.394	207.100
Body protein content—total in gm.	23.546	26.245	31.518	31.594	29.885
Maternal organism [2]					
Weight in gm.	150.000	166.203	194.476	195.742	176.620
Protein content in gm.	23.506	24.331	28.624	29.065	27.379
Young per mother					
Weight in gm.	.....	18.090	27.057	24.380	22.231
Protein content in gm.	.....	1.247	2.072	1.726	1.684
Kidney					
Weight in gm.	0.995	0.958	1.114	1.217	1.296
Protein content in gm.	0.161	0.153	0.177	0.193	0.205
Heart					
Weight in gm.	0.519	0.538	0.623	0.616	0.598
Protein content in gm.	0.083	0.089	0.103	0.103	0.098
Liver					
Weight in gm.	6.567	7.400	8.835	9.565	8.713
Protein content in gm.	1.256	1.307	1.602	1.838	1.727
Alimentary tract etc.					
Weight in gm.	25.751	30.959	32.209	35.060	29.552
Protein content in gm.	1.631	1.693	1.809	1.954	1.802
Blood clot					
Weight in gm.	2.452	2.235	2.545	2.875	2.551
Protein content in gm.	0.712	0.608	0.714	0.793	0.730
Blood serum					
Weight in gm.	2.876	3.213	3.450	3.520	3.160
Protein content in gm.	0.166	0.148	0.185	0.195	0.172
Uterus etc.					
Weight in gm.	0.290	7.040	10.768	8.272	8.249
Protein content in gm.	0.040	0.667	0.822	0.803	0.822
Carcass					
Weight in gm.	110.550	116.900	145.700	142.889	130.750
Protein content in gm.	19.497	20.333	24.034	23.989	22.645

[1] Definitions of organs and tissues used in this study, with the exception of that for "maternal organism protein," are given in Addis, Lee, Lew and Poo ('40).

[2] Total body weight or protein minus the weight or protein of uterus and embryos.

protein than the controls, i.e., the relation becomes one of a symbiosis in which the mother gains. The curves for carcass protein (mainly muscle, skin and skeleton) repeat the same story, but the loss on a low protein diet is greater and the gain with an adequate protein intake less.

The curves for organ protein, liver, kidney and heart show that each organ has its own individual mode of reaction to variation in protein intake. It is shown also that in the pregnant rat the liver has a relatively higher rate of anabolism than any other part of the strictly maternal body.

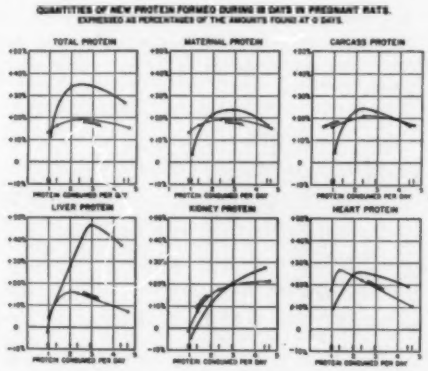

Fig. 1 The control curves are derived from data given in full in a recent number of this Journal (Addis, Lee, Lew and Poo, '40). These non-pregnant groups had the same initial body weights and were kept for 18 days on the same diets as the pregnant groups. The heavier lines are derived from the results in pregnant rats and were calculated from the data in table 1 of this paper.

The concentration of protein in the organs and tissues of pregnant rats (protein per 100 gm. organ or tissue weight) can be deduced from table 1. However, it is not the concentrations themselves but the question as to whether they are lower than the concentrations in the control non-pregnant groups that is of immediate interest to us. This relation is given in table 2 where the concentrations in pregnancy are expressed as + or — deviations from the concentrations in the controls.

The results given in table 2 show that the low concentrations we found in our previous work on a 16% protein diet were not due to a relative deficiency in protein consumption since low concentrations are still found with an entirely adequate protein intake and occur even when such an excess of protein is taken that the rate of anabolism of both mother and embryos is inhibited. Though the concentrations fall to their lowest level on a diet deficient in protein, this is only an accentuation of what is a constant characteristic of the state of pregnancy under all conditions. It remains true, of course,

TABLE 2

Concentration of protein in pregnancy and in non-pregnant rats after estradiol relative to the concentration in non-pregnant controls

ORGAN	PREGNANT RATS				NON-PREGNANT ESTRADIOL-TREATED RATS
	Dietary protein in per cent				Dietary protein in per cent
	11%	16%	27%	43%	16%
Serum	—39%	—20%	—16%	—20%	+8%
Alimentary tract etc.	—20%	—7%	—4%	—9%	+18%
Total maternal body	—11%	—5%	—6%	—5%	+4%
Carcass	—6%	—4%	—4%	—5%	+4%
Blood clot	—3%	—3%	—5%	—7%	—11%
Liver	—4%	—5%	—3%	—4%	+8%
Kidney	—4%	±0%	—2%	—7%	—2%
Heart	±0%	—2%	+4%	—4%	±0%

that no precise meaning can be attached to a decrease in protein concentration in material that consists of fat, minerals and water as well as protein. Inasmuch as serum and blood clot contain little fat and the mineral content is nearly constant, it is likely that a decrease in protein concentrations means dilution with water. Since there is no apparent increase in visible fat in pregnant rats, an increase in water content may also be the reason for the low concentrations in other tissues and organs. In any case this is a singular phenomenon for we have not found it under a variety of conditions in which pronounced changes in metabolism were induced by diet, thyroidectomy or thyroxin administration (Addis, Karnofsky,

Lew and Poo, '38). It is natural, therefore, to look for some circumstance peculiar to the pregnant state. One of these is the presence in the blood stream of relatively high concentrations of estrogenic substances.

We attempted to duplicate this peculiarity of pregnancy by injecting estradiol dipropionate into non-pregnant rats. All the other conditions of our present experiments were observed. Twenty rats were selected whose average body weight was 150 gm. on the day on which the diet containing 16% of protein was first given. They were killed 18 days later after having received 0.05 mg. of estradiol intramuscularly every other day, the last injection being given on the fifteenth day. Though the animals seemed to be quite healthy, their growth rate was slowed so that on the eighteenth day the average body weight was only 156 gm. In the last column of table 2 the protein concentrations are given expressed as a percentage of the concentrations in the control group on the same diet. Instead of being lower they are on the whole higher, a difference probably due to the fact that the animals in the estradiol-injected group were obviously leaner than the controls. There is no reason to suppose that the material we injected was ineffective, for the uterus, after the removal of the fluid it contained, weighed more than twice as much as that of the controls. When a single injection was given Zuckerman, Palmer and Bourne ('39) found an increase in the water content of the skin, and Thorne and Engel ('38) a decrease in sodium chloride and water excretion. It is possible that in our experiment with repeated injections over a period of 15 days, an equilibrium in salt and water exchange had been reestablished. In any event this experiment gives no support for the hypothesis that the lowered protein concentrations in pregnancy are due to the increase in estrogenic substances. We do not suppose that this hypothesis is thereby invalidated. It is obvious that we did not reproduce the actual hormone conditions that exist in the pregnant rat.

The distribution of protein (organ protein per 100 gm. total protein) has none of the ambiguity involved in measurements

of protein concentration in the body. Taking the total protein as representing 100%, this measure is a statement of the percentage assignment of the total available quantity among the various organs and tissues of the body. These organs and tissues each make their own demands. The proportion of the total they actually receive may be taken as evidence of their relative importance to the total economy. In pregnancy the total demand for amino acids for protein synthesis is increased and a new element is introduced by reason of the special requirements of the enlarging uterus and embryos. In pregnant animals subjected to a variation in food protein supply from amounts barely sufficient for maintenance to quantities in excess of all needs, we must consider changes in

TABLE 3

Distribution of protein in non-pregnant and pregnant rats

	NON-PREGNANT CONTROLS ON DIETS WITH PROTEIN CONCENTRATIONS IN PER CENT				PREGNANT RATS ON DIETS WITH PROTEIN CONCENTRATIONS IN PER CENT			
	11%	16%	27%	43%	11%	16%	27%	43%
	%	%	%	%	%	%	%	%
Uterus and young	0.2	0.2	0.2	0.2	7.3	9.2	8.0	8.4
Carcass and blood	88.0	87.5	87.4	87.3	80.3	79.1	79.1	78.8
Maternal organs	11.8	12.3	12.4	12.5	12.4	11.7	12.9	12.8
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

the distribution of protein if we desire to comprehend the effect of these conditions on the intermediary metabolism of protein within the body. The principal results are given in table 3 with the data for the controls.

In table 3 are presented our data with respect to three divisions of the body, namely, the uterus and its contents, the carcass and blood, and the maternal organs. The latter division includes all the abdominal and pelvic organs except the uterus. When such a gross division is made the results are clear. The proportion of protein allocated to the maternal organs remains substantially constant whether the animal is or is not pregnant and no matter what quantity of protein is consumed. The increase in the proportion of the total protein

that is allocated to the uterus and young is taken from the carcass and blood. Toward the end of pregnancy the amount of protein distributed among muscle, skin, skeleton and blood is diminished, but only relatively, for, as figure 1 shows, with an optimal supply of food protein the carcass protein is absolutely increased.

These are the main outlines of the effect of pregnancy on the distribution of body protein but there are subsidiary changes concealed by this method of presentation. Figure 1 shows that the absolute amount of protein laid down in the liver of the pregnant rat is out of proportion to the quantities formed by the livers of non-pregnant rats and that the gain is relatively greater in the liver than in the kidney or heart.

TABLE 4

Distribution of maternal organ protein in pregnancy relative to the distribution in non-pregnant controls

ORGAN	DIETARY PROTEIN CONCENTRATION IN PER CENT			
	11%	16%	27%	43%
Liver	+ 3%	+ 5%	+ 8%	+12%
Kidney	— 6%	— 7%	—13%	— 7%
Heart	—11%	—10%	—12%	— 5%
Alimentary tract etc.	— 1%	— 2%	— 4%	— 9%

We have seen that the proportion of the total protein allocated to the maternal organs is relatively constant. Within this constant organ proportion there may be differences in distribution of protein to the various organs that together make up the whole organ quantity. If this is the case the differences will be revealed by taking the whole maternal organ protein as 100% and ascertaining how this total amount is apportioned. The organ distributions in pregnancy relative to the distributions in the controls are given in table 4.

Table 4 shows that at all levels of protein intake the proportion assigned to the liver is greater than in the controls in contrast to the other organs where it is less. This, then, is a constant peculiarity of pregnancy. This is true whether the proportion of protein in the liver is calculated on the basis of

the total protein, of the total maternal protein, or of the maternal organ protein, though the difference, of course, is less marked if the total protein is considered as the unit. It is a difference that becomes more pronounced as the quantity of protein consumed as food increases. We are inclined to regard the phenomenon as an example of a work hypertrophy of the liver during pregnancy though we have no idea as to the nature of this work beyond the supposition that it is connected with protein metabolism and perhaps particularly with protein anabolism.

SUMMARY

1. The total quantity of protein formed during pregnancy is dependent on the protein intake. At low levels the quantity newly formed is even less than that of non-pregnant controls but with increasing protein intake it rises high above the control values and after attaining a maximum decreases as unusually large amounts of protein are consumed. The protein added to the mother's body (total protein less the protein of the uterus and its contents) is considerably less than that of non-pregnant rats when the protein intake is reduced to a gram per day, but when 2 or 3 gm. are eaten daily, the body of the pregnant rat gains more protein than the bodies of controls. In the organs the main effect of pregnancy is a pronounced increase in the protein of the liver at moderate and high levels of protein consumption.

2. The concentration of protein in the organs and tissues of pregnant rats is lower than that in non-pregnant rats at all levels of protein consumption. An attempt to reproduce in non-pregnant rats part of the hormone conditions of the pregnant state by the injection of estradiol propionate failed to support the hypothesis that the lowered protein concentrations were due to an increase in estrogenic substances.

3. At the end of pregnancy the gross distribution of protein in the body (organ or tissue protein per 100 gm. total protein) is not appreciably influenced by the amount of protein that has been taken as food. Roughly 8% of the total protein is

assigned to the uterus and embryos, 80% to the carcass and blood and 12% to the internal organs. All of the protein of the uterus and its contents represents that deflected from the carcass and blood which, in controls, accounts for 88% of the total. The proportion allocated to the internal organs is no different from that found in non-pregnant rats.

4. Although the proportion of the total protein assigned to the internal organs is about the same as the proportion found in non-pregnant animals the distribution among the various organs is different. In pregnant rats the liver obtains more protein and the other organs less. The preponderance of liver protein increases with increase in protein consumption.

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